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Hyperpolarization activated cyclic nucleotide gated (HCN) channels play an important role in limiting temporal summation and in setting the membrane potential of many cells in the brain. Their subcellular distribution is tightly regulated by an auxiliary subunit, Tetratricopeptide repeat-containing Rab8b interacting protein (TRIP8b). Interestingly, TRIP8b is extensively spliced and nine distinct isoforms exist. These isoforms have distinct (in some cases opposing) effects on the surface trafficking of HCN channels. Two distinct promoters (termed 1a and 1b for their first exons) lead to the expression of TRIP8b. Although 1a containing isoforms are known to be expressed in neurons, 1b isoforms are not. Moreover, knockout mice lacking the 1b isoforms show intact neuronal HCN channel function suggesting that the 1b isoforms are exclusively expressed elsewhere. Recent work has identified the expression of TRIP8b isoforms containing 1b in mature oligodendrocytes. Here, we extend this work by characterizing the developmental regulation of TRIP8b isoforms in the oligodendrocyte lineage using biochemical and electrophysiological approaches. We find that 1b isoforms are only expressed in oligodendrocytes and that they are developmentally upregulated coincident with the onset of myelination. Additionally, we note that mature oligodendrocytes express 1b isoforms in addition to the 1a isoforms expressed by neurons.
Abstract: Exposure to excess glucocorticoids during fetal development has long-lasting physiological and behavioral consequences, although the mechanisms are poorly understood. The impact of prenatal glucocorticoids exposure on sexually dimorphic stress responses in juvenile and adult offspring implicates the developing hypothalamus (HT) as a target of adverse prenatal glucocorticoid action. To identify glucocorticoid receptor (GR) target genes, primary cultures of male or female HT-neural progenitor/stem cells (NPSCs) were treated with vehicle or the synthetic glucocorticoid dexamethasone (Dex, 100nm) for 4h and the transcriptome determined using RNA-Seq. Bioinformatic analysis identified relatively high levels of a number of genes regulating stem cell proliferation and HT progenitor function. While several genes are regulated equivalently by Dex in male and female NPSCs, others show gender specific responses. Interesting, while these cells express GR, only low levels of sex-steroid receptors are expressed, suggesting that sex-specific differentially regulated genes identified are mediated by genetic and not hormonal influences at this age. Our studies provide the first characterization and description of sexually dimorphic glucocorticoid-regulated pathways in HT NPSCs and may reveal target genes responsible for the long-term consequences of fetal glucocorticoid exposure in adulthood.

Microrna-128 regulates proliferation and neurogenesis of neural precursors by targeting pericentriolar material 1 (pcm1) in the developing neocortex


Abstract: During the development of the embryonic neocortex, the tightly regulated expansion of neural progenitor cells (NPCs) and their differentiation into neurons are critical for normal cortical formation and function. The failure of these processes is associated with various neuropsychiatric disorders including autism spectrum disorders (ASDs). However, the molecular mechanisms that regulate NPC proliferation and differentiation remain unclear. In this study, we demonstrate that microRNA (miR)-128, which has previously been shown to be misregulated in ASDs, regulates the proliferation and differentiation of NPCs by repressing pericentriolar material 1 (PCM1). Specifically, the ectopic overexpression of miR-128 reduces proliferation but promotes NPC differentiation into neurons both in vivo and in vitro. Overexpression of miR-128 suppressed the translation of PCM1, and the knockdown of endogenous PCM1 phenocopied the observed effects of miR-128 overexpression on NPCs. Furthermore, the concomitant overexpression of PCM1 and miR-128 in NPCs rescued the phenotypes associated with miR-128 overexpression, enhancing neurogenesis but inhibiting proliferation in vitro and in utero. Taken together, these results demonstrate a novel mechanism by which miR-128 regulates the proliferation and differentiation of NPCs in the developing neocortex.

Abstract: Microcephaly – reduced head circumference as a result of decreased brain size – is an important cause of developmental disability. Microcephaly is highly genetic and identifying and characterizing microcephaly genes has provided many new insights into the genetic mechanisms of neurogenesis. We have previously reported that loss of function mutations in CHMP1A cause microcephaly with pontocerebellar hypoplasia and short stature. CHMP1A encodes a member of the ESCRT complex, a protein complex required for cellular functions including activated receptor degradation, exosome secretion, and cytokinesis. To understand the mechanism of CHMP1A in normal brain development we have generated a Chmp1a gene trap (GT) mouse line and CHMP1A null human induced pluripotent stem cell (iPSC) lines. Homozygous Chmp1a GT mice show early postnatal lethality and reduced body and brain size by late embryogenesis with abnormal development of the cerebral cortex and cerebellum. By late embryogenesis, whole brain mass is reduced 10% in GT embryos compared to controls. At E17.5, Chmp1a GT mice have reduced cortical plate thickness, with greater reduction of late-born upper layers than of early-born deep layers. At P0, Chmp1a GT mice have reduced cerebellum size, a thin external granule layer, and fewer proliferating granule cell precursors, defects that resemble disrupted Sonic hedgehog signaling. Together, these findings suggest that Chmp1a is required for brain development through regulation of cortical and cerebellar progenitor cell proliferation. RNA sequencing of neural rosettes differentiated from human iPSCs provides further evidence for
CHMP1A’s role in progenitor proliferation. We used RNA sequencing to measure global gene expression differences between 5-week-old CHMP1A null and control neural rosettes. Gene ontology (GO) term enrichment analysis identified clusters “mitotic cell cycle” and “regulation of neurogenesis” as significantly down regulated in the absence of CHMP1A and cluster “synaptic transmission” as significantly up regulated. In addition, the expression of several canonical markers of proliferating cortical progenitors was greatly reduced in mutant rosettes. These results provide further evidence that progenitor proliferation is disrupted in the absence of CHMP1A; this mechanism likely causes microcephaly in CHMP1A null patients and Chmp1a GT mice.


Poster

472. Molecular Mechanisms of Proliferation

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 472.05/A5

Topic: A.01. Neurogenesis and Gliogenesis

Support: Brain Mapping by Integrated Neurotechnologies for Disease Studies (Brain/MINDS)

Title: Analysis of the Gpr56 e1m-EGFP transgenic marmoset

Authors: *A. MURAYAMA*1,2, J. OKAHARA1, B. BYOUNG-IL4, C. A. WALSH4, E. SASAKI3, H. OKANO1,2; 1Marmoset Neural Architecture, RIKEN, BSI, Wako, Japan; 2Keio Univ. Sch. of Med., Tokyo, Japan; 3CIEA, Kawasaki, Japan; 4Harvard Med. Sch., Boston, MA

Abstract: G-protein coupled receptor 56 (Gpr56) has been reported as a cortical malformation-related gene that is mutated in patients, leading to a distinctive phenotype termed bilateral frontoparietal polymicrogyria. Recent study (Bae et al., Science 2014) showed that a 15-base pair deletion mutation in a regulatory element of Gpr56 causes polymicrogyria selectively surrounding the Sylvian fissure bilaterally. A minimal 300-bp Gpr56 e1m promoter contains the cis-regulatory element around this deletion region, and the Gpr56 e1m promoter-lacZ transgenic mice suggested that this Gpr56 e1m promoter region might be important of the Sylvian fissure formation. In addition, Gpr56 mRNA expressed especially in outer radial glial progenitor cells in human neocortex, which suggested that it may play an important role in gyrification. But mouse
is a lissencephalic animal and limited to study gyrification. To study how the minimal 300-bp Gpr56 e1m promoter functions during corticogenesis, we generated the Gpr56 e1m promoter-EGFP (enhanced green fluorescent protein) transgenic marmoset. The common marmoset, Callithrix jacchus, is a small New World Primate, which has clear Sylvian fissure, and we can now genetically engineer its embryo. We injected a lentiviral vector, carried Gpr56 e1m-EGFP, into marmoset embryos, and obtained two founder marmosets (F0). Using their fertilized embryos, we generated F1 animals. We performed immunohistochemistry of the cerebral cortex. Most of the EGFP-positive cells existed in the subplate (SP) and the cortical plate (CP). Some of them expressed progenitor markers and possessed basal processes like outer radial glia cells, but the cell bodies were not in the outer subventricular zone but in CP. It may suggest that Gpr56 e1m promoter did not work in the outer radial glia but played a role in the progenitors migrating in the CP and other specific neurons in SP and CP to build the cerebral cortex.


Poster

472. Molecular Mechanisms of Proliferation

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 472.06/A6

Topic: A.01. Neurogenesis and Gliogenesis

Support: INSERM AVENIR

ANR

Desire

Fondation Bettencourt Schueller

FNSNF

Fondation Gianni Biaggi de Blasys

Title: Dissecting the neurodevelopmental role of the microtubule-associated protein EML1, mutated in subcortical heterotopia

Authors: *S. BIZZOTTO¹, F. PHAN DINH TUY¹, M. KIELAR², D. ROMERO¹, A. HOULLIER², G. CANALI², E. WELKER², A. HOUDUSSE², A. CROQUELOIS², F.
Abstract: Subcortical band heterotopia (SBH) is a cortical malformation associated with intellectual disability and epilepsy, and characterized by the presence of a band of heterotopic neurons located in the white matter below a grossly normal cortex. Mutations were identified in the microtubule-binding protein Eml1/EML1 in the spontaneous HeCo (‘Heterotopic cortex’) mouse mutant, a model of SBH, and in patients exhibiting giant ribbon-like heterotopia. HeCo mice have misplaced progenitors and ectopic proliferation, which are likely to be the primary causes of their heterotopia. The role of Eml1 in neurodevelopment has not been previously studied. We found that during the neurogenic period in the mouse cortex it is expressed in both progenitors and post-mitotic neurons. We aimed to explore Eml1’s function in progenitors. Our objectives were to search for Eml1 protein partners in mouse embryonic brain extracts and to study Eml1 association and role with respect to microtubules (MTs). Also, we wanted to perform cell biology studies in vitro and in vivo to study neuronal progenitor behaviour in the presence or absence of Eml1. We found that Eml1 shows a cell cycle-dependent localization, enriched along spindle MTs and at the midbody, suggesting a role in spindle function and/or in cytokinesis. Recombinant Eml1 partially co-localizes with MTs in epithelial cells and the purified protein binds directly to MTs in vitro. Proteomics studies identified a list of potential protein partners, and we are focusing our analysis on networks of proteins known to be involved in cell division. We knocked down the expression of Eml1 in wild-type E14.5 mouse embryos using in utero electroporation, specifically in radial glial cell progenitors. Reduced expression of Eml1 leads to loss of radial orientation and shift of somata to more superficial positions. Eml1 re-expression in knockdown and HeCo embryos rescues the position of progenitors. We are further exploring the consequences of Eml1 loss on MT dynamics and cell division. Protein partners are being assessed for their roles in these processes. These data will contribute to understanding mechanisms of progenitor constraint in the ventricular zone during corticogenesis, and add knowledge concerning the causes of heterotopia formation.

The axon guidance receptor neogenin maintains adherens junction integrity by promoting assembly of the actin cytoskeleton

Authors: *N. K. LEE, A. WHITE, K. FOK, H. COOPER; Queensland Brain Inst., Univ. of Queensland, Brisbane, Australia

Abstract: A fundamental process driving organogenesis in the vertebrate embryo is epithelial morphogenesis. Choreographed cell movements of simple epithelial sheets generate complex structures such as the neural tube, the precursor of the brain and spinal cord. Dynamic cellular behaviors including epithelial folding and lumen formation require the epithelial sheets to remain elastic but resilient in response to local cues. Epithelial integrity is maintained by specialized cadherin-mediated cell junctions called the zonula adherens (ZA), and the formation and stability of these junctions is wholly dependent on the indirect interactions between cadherin adhesion complexes and the circumferential actin cytoskeleton running parallel to the ZA (the actin ring). Specifically, the actin ring is essential in promoting junctional tension mediated through actomyosin contractibility, and failure to regulate actin dynamics such as turnover leads to loss of adhesion, ZA collapse and epithelial disintegration. Thus, the molecular pathways that promote actin nucleation specifically within the spatial confines of the ZA are crucial factors mediating the fidelity of organogenesis and nervous system development. Neogenin, traditionally known as an axon guidance receptor for both Netrin-1 and Repulsive Guidance Molecules (RGMs), is essential for key embryonic processes. Depletion of Neogenin or RGMa in the neuroepithelium of the early neural tube leads to the inability to establish apicobasal polarity due to a loss of adhesion and ultimately a failure in lumen formation(1). Using the established Caco-2 cell model for studying cadherin-based cell-cell adhesion in epithelial cells, we demonstrate that loss of Neogenin via siRNA knockdown prevents cadherin homophilic adhesion between adjacent cells, leading to a severe disruption of junctional contacts. Additionally, we show loss of junctional integrity in Neogenin knockdown cells is due to a significant decrease in actin turnover which leads to dramatic changes in junctional tension. Furthermore, loss of Neogenin also results in reduced G-actin incorporation into growing actin filaments at the junction. Together, these findings suggest a new unidentified role for Neogenin in epithelial stability and junctional maintenance by regulating cadherin-mediated adhesion and modulating junctional tension via actin nucleation. This role may provide an explanation into the neural tube defect observed due to the loss of Neogenin in the neuroepithelium. 1.Kee, N., et. al. (2008). J. Neurosci. 28, 12643-12653.

Disclosures: N.K. Lee: None. A. White: None. K. Fok: None. H. Cooper: None.
Title: Role of rab18 in development and degeneration of cerebellum

Abstract: Rab GTPase proteins belong to the superfamily of Ras small G protein family. They coordinate multiple processes of membrane trafficking, including regulation of vesicle formation, transport, budding, tethering and fusion. In this study, we examined the physiological roles of Rab18 in vivo. Mutations of Rab18 are identified in patients with Warburg Micro Syndrome characterized by progressive limb spasticity, optic atrophy, hypoplasia of corpus callosum and cerebellum, suggesting Rab18 is involved in neurodevelopment. Recently, Rab18 is reported to be a candidate susceptibility gene of schizophrenia. Indeed, there is evidence for cerebellar pathology in schizophrenia, including decrease of Purkinje cells and reduction of cerebellar volume. In cultured cells, Rab18 is shown to cycle between cytosol and secretory granules and interact with secretory granules to repress exocytosis upon activation of the regulated secretory pathway, suggesting a role of Rab18 as a brake for secretion. Therefore, it is possible that Rab18 may control some secretory factors crucial for neurodevelopment. The Rab18-deficient mice showed severe hind limbs ataxia and reduced cerebellar size. We found that the foliation pattern of cerebellum was altered and the number but not the density of Purkinje cells was decreased at P28 in Rab18-deficient mice. At P7, the expression level of sonic hedgehog protein, the factor important for proliferation of granule cell precursors and secreted by Purkinje cells, was reduced in Rab18-/− cerebella. Yet the number of PH3-positive granule cell precursors in Rab18-/− cerebella was increased. Aberrant migration of developing granule cells was found as well. The processes of Bergmann glia, the crucial glia cells associated with migration of granule cells, were thickened in Rab18-/− mice. Furthermore, the expression level of
myelin basic protein, a myelination marker, and its distribution was lower and less complex but more condensed in Rab18−/− cerebella than in WT. In the 12-M old mice, the degenerating phenotypes of Purkinje cells were detected in Rab18−/− cerebellum, including decreased density of Purkinje cells, less organized dendritic arborization and degeneration of axonal terminal in deep cerebellar nuclei. In addition, gliosis was found in the internal granule cell layer and in Bergmann glia, and the expression of glial fibrillary acidic protein was significantly increased. However, no apoptotic evidence was detected at this age. Taken together, Rab18 is likely involved in the degeneration of Purkinje cells and proliferation and migration of granule cells during cerebellar development.

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

Poster

472. Molecular Mechanisms of Proliferation

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 472.09/A9

Topic: A.01. Neurogenesis and Gliogenesis

Support: Grant-in-Aid for Scientific Research (25460243), Japan Society for the Promotion of Science

Title: Role of microRNA in histogenesis of the cerebrum in mouse embryos

Authors: *R. HASHIMOTO1, I. KIHARA2, A. MATSUMOTO3, H. OTANI1;
1Dept. of Clin. Nursing, Fac. of Medicine, Shimane Univ., Izumo, Japan; 2Dept. of Sports and Hlth. Sci., 3Dept. of Developmental Biol., Fac. of Medicine, Shimane Univ., Izumo, Japan

Abstract: We searched the microRNAs (miRNAs) which were related to histogenesis of the cerebrum in mice and analyzed their roles in the development of the cerebral cortex. We extracted total RNA from the cerebra of mouse embryos from embryonic day (E) 12 to E15, and analyzed the change in expression of over 2000 miRNAs using RNA microarray assay. We chose the top five miRNAs that increased the expression and the top five miRNAs that decreased in that period, and measured expression levels of these ten miRNAs with quantitative real-time PCR method. From these results, we chose let7b-5p, which showed the most increased miRNA level from E12 to E15, and analyzed the expression pattern using technique of in situ hybridization. Let7b-5p was expressed around nuclei of the part of cells in the intermediate zone of the telencephalon on E12, and it was expressed all layers, but was stronger in the ventricular zone of the telencephalon on E15. Using RNA interference to analyze the function of let7b-5p,
we injected the double strand (ds) RNA of let7b-5p (DS group) which were labeled with fluorescein, and the ds RNA that has been confirmed not to affect the developmental process (control group) as the control treatment into the ventricle of mouse embryos on E13 using in-utero operation. We gave electrical stimulation at their heads for electroporation after injection. We collected the brains and measured their weight and size on E15. There was no significant difference in the brain size and weight between the DS group and the control group. However, roughness around the ventricle was observed, and irregularity of cell arrangement was detected around the cells in which the labeled dsRNA of let7b-5b was observed in the part of cerebral cortex by histological analysis. These results suggested that miRNA (let7b-5p) was involved in histogenesis of the cerebral cortex during embryonic period.

Disclosures: R. Hashimoto: None. I. Kihara: None. A. Matsumoto: None. H. Otani: None.

Poster

472. Molecular Mechanisms of Proliferation

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 472.10/A10

Topic: A.01. Neurogenesis and Gliogenesis

Support: 5 T32 GM007337

Title: NPAS1/NPAS3 regulation of DNA methylation in cortical inhibitory interneurons

Authors: *R. GENOVA¹, T. C. YIN², L. N. MCDANIEL², A. A. PIEPER¹;
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Abstract: Mutations in neuronal PAS domain proteins 1 and 3 (NPAS1 and NPAS3) have been identified in individuals with schizophrenia and autism spectrum disorders, but little is known about how these proteins contribute to disease pathogenesis. Mice deficient in NPAS1 and/or NPAS3 recapitulate behavioral and neurostructural features of major psychoses, and NPAS1 and NPAS3 were recently shown to reciprocally regulate the generation of inhibitory interneurons. Disrupted excitatory-inhibitory balance of cortical networks is thought to contribute to a number of neuropsychiatric conditions, and studies in patients with major psychoses have repeatedly shown aberrant DNA methylation patterns in inhibitory interneurons. Like NPAS1 and NPAS3, expression of the DNA methyltransferases DNMT1 and DNMT3a is primarily restricted to inhibitory interneurons. Because aberrant DNA methylation in inhibitory interneurons is associated with major psychoses, we are interested in investigating whether NPAS deficient mice
have similar epigenetic dysregulation. Preliminary data suggests that NPAS1 or NPAS3 deficiency alters cortical DNMT expression. We therefore hypothesize that NPAS1 or NPAS3 deficiency results in altered DNA methylation patterns via dysregulated DNMT expression in inhibitory interneurons. To characterize how the inhibitory interneuron methylome varies with NPAS1 and NPAS3 expression, we will utilize whole-genome bisulfite sequencing to identify DNA methylation patterns in inhibitory interneurons isolated via fluorescence activated cell sorting. Identifying regions of the genome with altered methylation patterns in NPAS1- or NPAS3-deficient animals may provide insight into epigenetic dysfunction in neuropsychiatric conditions.


Poster

472. Molecular Mechanisms of Proliferation

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 472.11/A11

Topic: A.01. Neurogenesis and Gliogenesis

Support: NEI-5R00EY019547

NINDS-5P30NS069266

Title: Understanding the role of minor spliceosome in mouse model for Microcephalic Osteodysplastic Primordial Dwarfism Type I

Authors: *R. KANADIA, M. BAUMGARTNER;
Univ. of Connecticut, Storrs, CT

Abstract: The importance of minor spliceosome in human development is underscored by the disease microcephaly osteodysplastic primordial dwarfism type I (MOPD-I), where the cardinal symptoms include, microcephaly and intrauterine growth retardation of limbs. The underlying molecular defect is that of compromised minor spliceosome function. The spliceosome executes splicing by base-pairing of small nuclear RNA (snRNAs) to consensus sequences flanking exon/intron boundaries. These consensus sequence are found in >99% of the introns in the mammalian genome and are recognized and processed by the major spliceosome consisting of U1, U2, U4, U5, and U6 snRNAs. However, there are divergent sequences at the exon/intron boundary that are found embedded within ~450 genes that cannot be recognized by the major spliceosome snRNAs. These minority introns are recognized and processed by the aptly named
minor spliceosome consisting of U11, U12, U4atac, U5 and U6atac snRNAs. The regulatory advantage afforded by the presence of a minor-intron in a gene that mostly consists of major intron is not clearly understood. Minor spliceosome snRNAs are expressed at a lower level than its major counterpart resulting in inefficient splicing and post-transcriptional damming effect to control protein production of these ~450 genes in the mammalian genome. Currently, there are no reports that define the role of minor spliceosome in mammalian development. To address this issue we have generated a U11 snRNA conditional knockout (cKO) mouse. While constitutive knockout is embryonic lethal (~E6), knockout of U11 in neural precursors via Emx1-Cre results in microcephaly at birth, which recapitulates the cardinal phenotype observed in MOPD-I patients. This allows us to deconstruct for the first time the molecular, cellular and behavioral deficits associated with MOPD-I in a mouse model. Currently, we are employing a custom microarray to interrogate en masse the successful splicing of minor introns in the developing U11-cKO brain, which will be complemented by RNAseq to interrogate transition in expression kinetics and minor intron splicing efficiency. In all, we report the recapitulation of microcephaly in U11-cKO mouse and will present the targets affected by loss of U11 and their function in regulating cell cycle of neural precursors that results in MOPD-I.

Disclosures: R. Kanadia: None. M. Baumgartner: None.

Poster

472. Molecular Mechanisms of Proliferation

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#/Poster#: 472.12/A12

Topic: A.01. Neurogenesis and Gliogenesis

Support: JSPS KAKENHI 24300134

Title: Necdin downregulates EGFR signaling in cortical neural precursor cells to suppress gliogenesis

Authors: *I. FUJIMOTO, K. HASEGAWA, K. FUJIWARA, K. YOSHIKAWA; Osaka university, Inst. for Protein Res., Suita, Japan

Abstract: Epidermal growth factor receptor (EGFR) is a receptor tyrosine kinase that controls the proliferation and differentiation of neural precursor cells (NPCs). Cortical NPCs committed to the astrocyte lineage express high levels of EGFR at late embryonic stages of mouse development. Necdin, a pleiotropic protein abundantly expressed in neurons and NPCs, suppresses cellular proliferation and apoptosis. We have previously shown that necdin interacts...
with the NGF receptor TrkA, another receptor tyrosine kinase, to enhance NGF signaling and promote survival of sensory neurons (J Neurosci, 25:7090-7099, 2005). In the present study, we examined whether necdin interacts with EGFR in primary NPCs and controls gliogenesis. Primary NPCs were prepared from mice at embryonic day 14.5 and cultured in the presence of bFGF for 4 days. Immunocytochemical data showed that necdin was expressed in NPCs and co-localized with EGFR in the cytoplasm after EGF treatment. Co-immunoprecipitation assay revealed that necdin bound to autophosphorylated EGFR but not to unphosphorylated EGFR. Necdin interacted directly with the tyrosine kinase domain (TKD) of EGFR, and deletion of the EGFR C-terminal domain enhanced the interaction, suggesting that the unphosphorylated C-terminal tail interferes with the interaction between necdin and the TKD. Necdin failed to affect EGF-dependent EGFR autophosphorylation in NPCs but reduced the interaction between EGFR and Grb2, an adaptor protein that activates the ERK1/2 pathway. In NPCs prepared from necdin-null mice, phosphorylated ERK1/2 levels were significantly increased. Furthermore, EGF-dependent astrocyte proliferation increased significantly in necdin-null NPCs treated with cardiotrophin-1. These data suggest that necdin controls EGFR signaling and suppresses EGFR-mediated glial proliferation during embryonic cortical development.


Poster

472. Molecular Mechanisms of Proliferation

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 472.13/A13

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH Grant R01NS082283

NIH Grant P20 GM103620

NIH Grant P20 GM103548

Title: Guanine exchange factors regulate the neurogenic niche

Authors: *J. T. Cain¹, S. Koh¹, D. Timm¹, S. Duncan¹, R. O’Toole¹, T. Samson², E. S. Wittchen², K. Burridge², J. M. Weimer³;
¹Sanford Res., Sioux Falls, SD; ²UNC Sch. of Med., Chapel Hill, NC
**Abstract:** Neurogenesis is a tightly orchestrated process and disruption of neural progenitor (NP) specification, migration, or differentiation has profound effects on the formation of the cortex. Radial Glia (RG) are specialized NPs with an apico-basal polarity consisting of radial processes making contact with both the ventricular and pial surfaces; these processes provide an instructive scaffold for the radial migration of neurons in the cortex. RG can undergo either a proliferative symmetric division resulting in two RG, or an asymmetric division resulting in one RG and either an immature neuron or intermediate progenitor (IP). The type of division is dependent upon the rearrangement of cytoskeletal machinery, which is controlled by members of the Par polarity complex and the Rho GTPase Cdc42. Rho GTPases, including Cdc42 and RhoG, serve as regulators of progenitor proliferation and fate determination. Rho GTPases are themselves regulated by guanine-nucleotide exchange factors (GEFs) and GTPase activating proteins (GAPs) which control the GTP-loading state of Rho GTPases. Because GEFs act as activators for Rho GTPases, we hypothesize that GEFs are critical to Rho GTPase-mediated regulation of neural progenitors through the neurogenic niche including their proliferation and migration in the developing cerebral cortex. To test this hypothesis we have focused on SH3-containing guanine exchange factor (SGEF): a GEF whose expression is restricted to defined neurogenic niches in the developing cerebral cortex, is an activator of RhoG and Cdc42, and whose deletion appears to have nominal consequences outside of the central nervous system. To examine proliferation and migration of neural stem cells SGEF-/- mouse embryos were harvested at key developmental time points, E15.5 and E18.5. Pregnant dams were pulsed with BrdU at either 1 or 72 hours prior to sacrifice. Proliferation of RG and IP were assessed with coimmunolabeling of Brdu and Pax6 or Tbr2. Cortical layer-specific markers were used to assess defects in cortical lamination. Our findings show that at E15.5, SGEF-/- mice have a reduced IP (Tbr2+) population while RG (Pax6+) are unaffected. By E18.5, the loss of SGEF results in reduced thickness of the cortical layers.


**Poster**

**472. Molecular Mechanisms of Proliferation**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#: 472.14/A14**

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

**Title:** Rhox8 expression in the developing and adult mouse brain and spinal cord
**Authors:** H. G. HUFFMAN¹, G. R. PRATHER², J. A. MACLEAN, II², †J. L. CHEATWOOD¹; ¹Anat., ²Physiol., SIU Sch. Med., Carbondale, IL

**Abstract:** Homeobox transcription factors govern many developmental events. The Rhox, (X-linked reproductive Homeobox)-, genes are a recently discovered set of homeobox genes. Characteristically, the Rhox genes exhibit a complex spatially and temporally regulated expression pattern which varies in cell type during testis development and postnatally. Of all 33 mouse Rhox genes, Rhox8 is unique because it is the only one in the set that shows expression in the somatic cells in the embryonic testis. Rhox8, Rhox5, and Sox9 were all found to be highly expressed in positive control adult mouse testis tissue. Because it is a common phenomenon that highly-expressed testis genes also exhibit expression in the brain, often via an alternatively spliced message that may or may not produce functional protein, we sought to characterize Rhox gene expression in mouse and rat brain. Of these only Rhox8 had moderate expression in mouse cortex. As expected, Rhox8 and Sox9 mRNA are expressed in the adult rat and mouse brain, albeit at much lower levels than in the testis and ovary. Also, we found that Rhox8 mRNA is statistically upregulated at time point E13.5 compared to other time points in the developing mouse embryo. Females may express Rhox8 two-fold higher than males at E13.5 (p=0.057; more research needed at the time of abstract submission). Two genes related to Rhox8 expression, Sox9 and Gata1 were both expressed in the developing mouse embryo at all time points. Sox9 was also found to be expressed in rat cerebellum in all samples. Rhox5 mRNA was not detected in adult rat cerebellum, developing mouse embryo, or adult mouse samples. Expression of RHOX8 protein was detected via fluorescent immunohistochemistry in E13.5 tissue and adult tissue but not in E15.5, E17.5, and P1. Further research will aim to determine the location of expression. Rhox8 is expressed in murine adult total brain, cerebellum, cortex, and spinal cord. Ongoing studies in mice aim to determine female and male differences in expression of Rhox8, why Rhox8 is upregulated during E13.5 time point, why there is upregulation in the spinal cord and cortex, and to determine the full length Rhox8 transcript in the mouse brain.

**Disclosures:** H.G. Huffman: None. G.R. Prather: None. J.A. MacLean: None. J.L. Cheatwood: None.

**Poster**

**472. Molecular Mechanisms of Proliferation**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 472.15/A15

**Topic:** A.01. Neurogenesis and Gliogenesis
Support: The Velux Foundation
The Foundation for Neurological Research
The Jascha Foundation
Hartmann Brothers Foundation
Torben and Alice Frimodts Foundation
Aase and Ejnar Danielsens Foundation

Title: Long-term survival of newly formed neurons in the adult rat hippocampus following electroconvulsive stimulation - a stereological study

Authors: *M. V. OLESEN*, G. WÖRTWEIN²,³, B. PAKKENBERG¹; ¹Lab. for Stereology and Neurosci., Bispebjerg Hosp., Copenhagen, Denmark; ²Lab. of Neuropsychiatry, Dept. of Neurosci. and Pharmacol., ³Section of Envrn. Health, Dept. of Publ. Hlth., Univ. og Copenhagen, Copenhagen, Denmark

Abstract: Objectives: We aimed to test the hypothesis that stress-induced depression and electroconvulsive stimulation (ECS) have opposing effects on hippocampal neurogenesis. Next, we wanted to examine if ECS leads to long-term survival of the newly formed neurons. Background: The neurobiological mechanisms underlying depression are not fully understood. One hypothesis states that hippocampal neurogenesis and neuronal differentiation may be important factors in the recovery from depressive episodes. Thus, opposing effects of depression and antidepressant treatment on neurogenesis have been found. However, most previous studies using semiquantitative or quantitative counting methods report conflicting data on the short-term effects on neurogenesis. Thus, studies using methods that represent state of the art in regard to stereological cell counting and research into the long-term survival of newly formed neurons is lacking. Methods: We used a validated rat model of depression, chronic restraint stress (CRS), in combination with a clinically relevant schedule of ECS. The behavioral effects of CRS and/or ECS were assessed in the forced swim test. To estimate neurogenesis, the total number of neurons and the volume of hippocampal subregions in rats surviving 24 hours as well as 3, 6 and 12 months we used a validated stereological cell counting method, the optical fractionator. Results: Our results show that CRS induces depression-like behavior, without significantly changing neurogenesis, the total number of neurons or the volume of the hippocampal subregions. Further, ECS prevents stress-induced depression-like behavior and acutely increases neurogenesis by ~250%. Approximately 60% of the new neurons survive for 3 months with a further decline to 50% following 6 and 12 months. One year after ECS, neurogenesis is significantly increased by ~150% compared to control. The total number of neurons and the granule cell layer (GCL) volume was not affected by ECS at any time point. Conclusion: We show that ECS increases hippocampal neurogenesis and that a significant part of the newly
formed neurons survive for at least 12 months. CRS do not changes neurogenesis, total number of neurons or volume of the hippocampus. Thus, previous findings of volumetric changes are not caused by cellular alterations in the GCL, rather they appear to be caused by alterations in other subregions and/or altered gliogenesis as well as angiogenesis.

**Disclosures:** M. V. Olesen: None. G. Wörtwein: None. B. Pakkenberg: None.

**Poster**

**472. Molecular Mechanisms of Proliferation**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program/#Poster#: 472.16/A16

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

**Support:** Italian Ministry of Research grant, FIRB Idea Progettuale 2005

Italian Ministry of Research grant, CNR Progetto d’Interesse Strategico Invecchiamento 2012-2016

**Title:** Gpr37l1-Ptch1 interaction in the murine cerebellum during postnatal development and adulthood

**Authors:** *D. MARAZZITI, C. DI PIETRO, G. LA SALA, Z. ABBASZADEH, R. MATTEONI, G. P. TOCCHINI-VALENTINI; Inst. of Cell Biol. and Neurobio., Monterotondo Scalo, Italy

**Abstract:** Primary cilia are slender plasma membrane protrusions, found in most mammalian cell types. They crucially sense, integrate and transduce physico-chemical extracellular stimuli and are modelled as performing Bayesian inference of cell’s environment. Increasing evidence indicates that primary cilia are key coordinators of tissue- and cell type-specific signaling pathways during development and in tissue homeostasis and, when defective, are a major cause of human diseases and developmental disorders. Ciliary functions are, in particular, required for cerebellar differentiation and Shh-dependent proliferation of neuronal granule precursors (GCPs). The mammalian G-protein coupled receptor 37-like 1 (Gpr37l1) is specifically expressed in cerebellar Bergmann glia astrocytes and participates in the regulation of postnatal cerebellar granule neuron proliferation-differentiation, Bergmann glia and Purkinje neuron maturation. The Gpr37l1 protein interacts with the patched 1 (Ptch1) component of the primary cilium-associated, sonic hedgehog (Shh) receptor complex. We are investigating the functional
interaction between Gpr37l1 and Ptch1, as modulating the Shh-Ptch1-smoothened mitogenic pathway in primary cilia of murine cerebellum, during postnatal development and adulthood.

**Disclosures:**
- D. Marazziti: None.
- C. Di Pietro: None.
- G. La Sala: None.
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- R. Matteoni: None.
- G.P. Tocchini-Valentini: None.

**Poster**

472. Molecular Mechanisms of Proliferation

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 472.17/A17

**Topic:** C.04. Neurodegenerative Disorders and Movement Disorders

**Support:** Youming LU

Qing Tian

**Title:** Transgenic inhibition of synaptic transmission of new born neurons in dentate gyrus impairs learning and memory

**Authors:** *N. Tang;* Tongji Med. Col. Huazhong Univ. and Tec, Hubei, China

**Abstract:** The hippocampus plays an important role in learning and memory. The dentate gyrus (DG) subfield exhibits continued neurogenesis till adulthood. In the present study, we developed a generally applicable tetanus toxin (TeTX)-based method to generate transgenic mice, which permits inducible and reversible inhibition of synaptic transmission by restraining VAMP2 in the DG new born neurons (DGNBN). We found that blue light stimulating ChR2 retrovirus infected DGNBN induced EPSC in DG and CA3, but inhibition of synaptic output in DGNBN showed no response to blue light illumination. Moreover, morris water maze test and fear conditioning results indicated impaired hippocampus-dependent learning and memory in the above VAMP2 reduced transgenic mice. Our results demonstrated that DGNBN can integrate into preexisting circuits, and participate in the functions of learning and memory.

**Disclosures:** N. Tang: None.

**Poster**

472. Molecular Mechanisms of Proliferation
**Abstract:** The Mediator complex has emerged as a conserved regulator of gene transcription from yeast to human. It is part of the general transcription machinery and acts as a bridge between transcription regulators and RNA polymerase II. It has a large core module and a small detachable CDK8 module, which consists of four proteins: Med12, Med13, Cyclin C and Cyclin dependent kinase 8 (Cdk8). Mutations in the CDK8 module subunits have been connected to various forms of cancer and neurodegenerative diseases. It has been shown that Alzheimer’s patients had elevated RNA levels of cyclin C in their neurons and glial cells. Mutations in the med12 gene are found in patients with intellectual disabilities, such as Opitz-Kaveggia (FG) syndrome, Lujan syndrome and Ohdo syndrome (Maat-Kievit-Brunner type). As the function of the Cdk8 module is so crucial in several cellular and developmentally important processes and has a growing medical importance, we set out to study the role of the Cdk8 module to cellular reproduction and differentiation in the simple eukaryote fission yeast. We have found that the control of Cdk8 activity is responsible for the timing of mitotic commitment, therefore is part of a control network that regulates cell division. Our present findings indicate that the Cdk8 activity is regulated by the Cyclin-CDK interaction in vivo, regardless of its connection to the Mediator complex. We also show that Cdk8 might be interacting with other cyclins when it is not bound to the core module. As the CDK8 module has a deep evolutionary origin and is highly conserved among diverged eukaryotes, we believe that our observations can help to understand the role of the CDK8 module in higher eukaryotes.

**Disclosures:** G. Banyai: None. Z. Szilagyi: None. C.M. Gustafsson: None.

**Poster**

472. Molecular Mechanisms of Proliferation

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM
**Program#/Poster#:** 472.19/A19

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

**Support:** NIH Grant EY022030-03

**Title:** Heparin Binding-Epidermal Growth Factor (HB-EGF) stimulates the proliferation of Müller glia-derived progenitor cells in avian and murine retinas

**Authors:** *L. VOLKOV, A. FISCHER, L. TODD;
Ohio State Univ., Columbus, OH

**Abstract:** Müller glia can dedifferentiate and form proliferating Müller glia-derived progenitor cells (MGPCs) with the capacity to regenerate retinal neurons. Heparin Binding-Epidermal Growth Factor (HB-EGF) may play a key role in initiating formation of proliferating MGPCs.
HB-EGF has been demonstrated to be necessary and sufficient for HB-EGF formation in the zebrafish retina (Wan, et al. 2012). Currently, there is no information regarding the influence of HB-EGF in the regeneration of avian or mouse retina. We found that both hb-egf and egfr were rapidly and transiently increased following NMDA excitotoxic damage in the avian retina. In situ hybridization data suggests hb-egf is produced by Müller glia following retinal damage. In undamaged retina, intraocular injection of HB-EGF did not stimulate proliferation or downstream signaling pathways of canonical HB-EGF/EGFR signaling in either the chick or mouse retina. However, when paired with NMDA, HB-EGF was able to stimulate the proliferation of MGPCs in both avian and murine retina. Immunohistochemical data suggests that HB-EGF treatment following retinal damage stimulated the expression of the MAPK signaling components pERK and pCREB in Müller glia. Additionally HB-EGF stimulated pS6 signaling in Müller glia suggesting activation of mTOR signaling. Additionally, inhibition of EGFR reduced the proliferation of MGPCs in the avian retina after retinal damage. We conclude that HB-EGF plays a key role in the proliferation of MGPCs in both the chick and mouse retina and can be a considered a key target for stimulating the regenerative potential of MGPCs in the higher vertebrate.

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

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**Program#/Poster#:** 473.01/A20

**Poster**

473. Role of Adhesion in the Development of Neuronal Wiring

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM
**Topic:** A.04. Axon and Dendrite Development

**Title:** Directing neurite growth in collagen gel 3-dimensional neuronal culture

**Authors:** M. ANTMAN-PASSIG¹, K. BARANES¹, S. LEVI¹, *O. SHEFI²;  
¹Fac. of Engin. and Inst. of Nanotechnology and Advanced Materials, Bar Ilan Univ., Ramat Gan, Israel; ²Fac. of Engin. and Inst. of Nanotechnologies and Advanced Materials, Ramat Gan, Israel

**Abstract:** The ability to manipulate and direct neuronal growth has great importance in the field of tissue engineering, both for neuronal repair and potential medical devices. In-vivo, neurons grow and develop neurites in a 3-Dimensional (3D) extra cellular matrix (ECM) surrounding. Thus, imitating the 3D environment within a natural material as collagen is most important to simulate in-vivo conditions. We designed and developed a method to grow neurons in a 3D environment. A collagen hydrogel system was chosen as a 3D ECM analog to best mimic the natural environment of cells. We used primary leech (Hirudo medicinalis) neuronal culture and followed the growth of single cells for up to 7 days. We compared the neuronal growth in 3D to a 2D model and showed that neurons grown in 3D collagen gels develop significantly longer dendritic trees and neurites, while number of neurites originating from cell soma was smaller in 3D collagen gels. To manipulate neuronal growth we developed a method to align collagen matrix via inducing strain on collagen gels. We showed fiber directionality by analysis of light microscope images via Fast Fourier transform and by SEM imaging. Finally, we evaluated leech neurite extension within aligned gels. Using this method we've directed neuronal growth coinciding with collagen matrix orientation. We found no significant change in neurite lengths in aligned gels compared to control gels, demonstrating that cell growth and behavior does not alter except direction of growth. We have also developed a tunable platform to direct neurite growth in 3D by embedding nanoparticles and magnetic elements in a collagen gels which operate as topographic cues to guide neurite growth. Both these methods present a promising neuronal repair system in a realistic environment.
Disclosures: M. Antman-Passig: None. K. Baranes: None. S. Levi: None. O. Shefi: None.

Poster

473. Role of Adhesion in the Development of Neuronal Wiring

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 473.02/A21

Topic: A.04. Axon and Dendrite Development

Support: NIH Grant 1R15DC010910-01.

Title: Lingual ephrin-A’s and ephrin-B’s repel embryonic geniculate and trigeminal neurites *in vitro*

Authors: R. W. TREFFY, D. CHO, M. L. RUSSO, *M. W. ROCHLIN;
Dept Biol, Loyola Univ. Chicago, Chicago, IL

Abstract: Taste axons of the geniculate ganglion innervate pre-gustatory epithelium in fungiform papillae with high fidelity, and somatosensory axons from the trigeminal ganglion innervate the neighboring epithelium. Diffusible factors such as neurotrophins and semaphorins have key roles in guiding these afferents, but non-diffusible cues are also likely to be involved. Ephs and ephrins are cell surface molecules that act as ligands and receptors for one another, initiating signaling cascades that can cause repulsion, stabilization, or growth promotion of
There are two classes of Ephs and ephrins: ephrin-A’s are lipid-linked proteins that interact predominantly with EphA’s, whereas ephrin-B’s are transmembrane proteins that interact predominantly with EphB’s. During intralingual axon targeting and target penetration in rats and mice, anti-ephrin-A3 labels the lingual epithelium, and anti-ephrin-A1 labels the transverse and longitudinal musculature. Antibodies against EphA5 and EphA7 label afferents within nascent fungiform papillae. To determine if ephrin-A’s are capable of guiding axons we grew explants of geniculate and trigeminal ganglia on coverglasses coated with stripes of ephrin-A-Fc fusion proteins. Ephrin-A1, -A2, -A3, and -A4 repel geniculate neurites dose dependently in vitro. Trigeminal neurites are also repelled by these ephrins, though the repulsion is less robust. Together, these data are consistent with a guidance role for ephrin-A’s during pathfinding and targeting of gustatory and somatosensory axons in the tongue. Ephrin-B2 is also expressed along the dorsal epithelium of the mouse and rat tongue by E15.5 and E18, respectively, just after axons penetrate the epithelium; and ephrin-B-Fc’s are repellent in vitro. Combining intermediate concentrations of ephrin-A-Fc’s and ephrin-B-Fc’s in the same stripe results in additive repellent effects.


Poster

473. Role of Adhesion in the Development of Neuronal Wiring

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 473.03/A22

Topic: A.04. Axon and Dendrite Development

Support: 1R21MH098463

Title: Examining the structural determinants of Protocadherin-19 function in brain morphogenesis and epilepsy

Authors: *S. R. COOPER¹, J. D. JONTES², M. SOTOMAYOR²; ¹Dept. of Neurosci., ²The Ohio State Univ., Columbus, OH

Abstract: Members of the cadherin superfamily mediate calcium-dependent cell-cell adhesion and mutations in them have been linked to multiple neurodevelopmental disorders. It is thought that selective adhesion mechanisms of various cadherin superfamily members may be important for proper neural circuit development. Much has been learned about the molecular mechanisms used by the subfamily of classical cadherins that include epithelial and neural cadherins (Ecad and Ncad). These classical cadherins interact tip-to-tip and exchange tryptophan residues that are...
essential for adhesion. Interestingly, non-classical cadherins lack this tryptophan, yet they still form adhesive complexes. Protocadherin-19 (Pcdh19) is a non-classical member of the cadherin superfamily, and previous work suggests that it forms complexes with a distinct adhesive mechanism. Specifically, Pcdh19 mediates homophilic adhesion when in complex with Ncad, yet this adhesion is mediated by Pcdh19 itself and is independent of the classical Ncad tryptophan exchange. In addition, diverse missense mutations within the Pcdh19 extracellular domains have been implicated in epilepsy and mental retardation limited to females (EFMR). These mutations are believed to disrupt Pcdh19 directed adhesion and in turn disrupt proper neural circuit development. Here I present adhesion assays indicating which extracellular cadherin (EC) repeats are critical for adhesion. Of the six EC repeats of Pcdh19, only EC1 to EC4 are necessary; and of the five EC repeats of Ncad, EC1 to EC2 are sufficient to support adhesion of the Pcdh19-Ncad complex. We also present the first crystal structures of Pcdh19, including EC1 to EC4 at 3.5 Å, EC2 to EC4 at 2.7Å, and EC3 to EC4 at 2.5Å resolution. These structures show potential dimer interfaces that could contribute to adhesion and provide a structural frame work to infer the biochemical and biophysical consequences of EFMR mutations on Pcdh19 function.

Disclosures: S.R. Cooper: None. J.D. Jontes: None. M. Sotomayor: None.

Poster

473. Role of Adhesion in the Development of Neuronal Wiring

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 473.04/A23

Topic: A.04. Axon and Dendrite Development

Support: NEI R01 EY015788

NEI T32 EY022312

Title: The role of Down syndrome cell adhesion molecule in mouse retinofugal circuit development

Authors: *T. J. BURBRIDGE1, A. M. GARRETT2, Y. LI1, T. L. SPENCER-SALMON1, M. R. GRACE1, E. STEIN1, R. W. BURGESS2, M. C. CRAIR1;
1Neurobio., Yale Univ., New Haven, CT; 2The Jackson Lab., Bar Harbor, ME

Abstract: Down Syndrome Cell Adhesion Molecule (DSCAM) is involved in many processes in neural development including axonal guidance and branching, cell and neurite self-avoidance, and developmental cell death. Following reports of an enlarged midbrain and disrupted retinal
and retinogeniculate circuits in DSCAM knockout (KO) mice, we investigated the role of DSCAM in visual circuit development in the superior colliculus (SC). We found that retinocollicular projections in Dscam KO exhibited disrupted retinal axon laminar targeting, enhanced eye-specific segregation, and a striking axon “clustering” phenotype that disordered retinotopic continuity throughout visual SC in a regular “honeycomb” pattern. These circuit disruptions were developmentally regulated, interestingly becoming evident only after normal critical periods of refinement in the SC are complete. Using region-specific Dscam conditional knockout (cKO) mice, we also found that axon clumping and enhanced segregation were controlled in a region-specific manner by retinal DSCAM, while midbrain size and laminar mistargeting were specifically dependent on midbrain DSCAM. Further analysis of ‘honeycomb’ RGC axon clusters in the SC revealed that single retinocollicular axons in retinal Dscam cKO mice tended to respect interior and exterior cluster boundaries, and that the cell bodies of SC neurons were reorganized around RGC axon clusters in a pattern that resembled “barrels” in the somatosensory system. SC phenotypes caused by conditional Dscam deletion in the retina also depended on spontaneous activity, with disrupted early postnatal retinal spontaneous activity patterns largely nullifying the effects of retinal Dscam cKO on retinocollicular projections, although recordings of patterns of spontaneous activity in the SC were surprisingly normal until the age when anatomical (and physiological) clustering became apparent. Moreover, Bax KO mice, which have similar effects in the retina as Dscam KO mice, fail to show any retinocollicular axon phenotypes. We are currently testing the hypothesis that defects in Dscam KO retinal organization cause downstream circuit disruptions through activity-dependent refinement, with the potential concurrent necessity of DSCAM in retinal ganglion cell axons for axon self-recognition, avoidance, or branching during visual circuit development.

**Disclosures:** T.J. Burbridge: None. A.M. Garrett: None. Y. Li: None. T.L. Spencer-Salmon: None. M.R. Grace: None. E. Stein: None. R.W. Burgess: None. M.C. Crair: None.

**Poster**

473. Role of Adhesion in the Development of Neuronal Wiring

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program/#/Poster#:** 473.05/A24

**Topic:** A.04. Axon and Dendrite Development

**Support:** KAKEN 15K06770

**Title:** Glycoprotein M6a regulates laminin-inducing rapid determination of polarity in the cortical neuron
**Authors:** *A. Honda*¹,², Y. Ito¹, M. Igarashi¹,²;  
¹Grad. Sch. of Med. and Dent. Sci, Niigata Univ., Niigata, Japan; ²Ctr. for Transdisciplinary Res, Niigata Univ., Niigata, Japan

**Abstract:** Determination of the neuronal polarity plays a critical role in the brain development. However, at that process, the protein components in the plasma membrane, which mediates between the extracellular and the intracellular signals, have not been established well. We have previously reported that RNAi inhibition of glycoprotein M6a, one of the most abundant proteins in the growth cone membrane by our proteomic studies (Nozumi et al., PNAS, 2009), suppressed the axon formation on laminin. We also found that M6a formed a ternary complex with M6a-binding protein (M6BP) and its interacting protein, active formed Rap2. Here, we examined the relationship between this ternary complex and the laminin-induced polarity in the cortical neurons. According to the definition by Dotti et al. (J Neurosci, 1988), one of several neurites was finally specialized to an axon on 1-2 days after plating. However, on laminin, we found that an axon-like neurite selectively protruded at only a few hours after the plating. The M6a-ternary complex showed the asymmetric localization at the stage1 neuron on laminin, although at that stage, other well-known polarity determinants (e.g. Par3, CRMP2, and so on) remained to be still symmetrically distributed. In the M6a- or M6BP-knockout neurons, the rapid determination of the polarity by laminin was inhibited. These results indicated that the extracellular laminin signal accelerated the polarity determination of the cortical neurons through the M6a-ternary complex. shRNA knockdown experiments of M6a showed that suppression of M6a expression in the embryonic cerebral cortices inhibited morphological transition of migrating cortical neuron from multipolar to mono-(bi-) polar in the intermediate zone (IZ). We concluded that the above *in utero* results are consistent with our *in vitro* ones and that the M6a-ternary complex physiologically participates in the accelerated polarization.

**Disclosures:** A. Honda: None. Y. Ito: None. M. Igarashi: None.

**Poster**

473. Role of Adhesion in the Development of Neuronal Wiring

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program/#/Poster#: 473.06/A25

**Topic:** A.04. Axon and Dendrite Development

**Support:** NSF (IOS-1354898)

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NIH S10 Instrumentation Grants S10RR027303
NIH Grant UL1
NCATS Grant TR000430

Title: Massive expansion of protocadherin genes in the octopus genome

Authors: *Z. Y. WANG¹, C. B. ALBERTIN², O. SIMAKOV³,⁴, T. MITROS⁵, D. S. ROKHSAR⁵,⁶, C. W. RAGSDALE¹;
¹Neurobio., ²Organismal Biol. and Anat., Univ. of Chicago, Chicago, IL; ³Okinawa Inst. of Sci. and Technol. Grad. Univ., Okinawa, Japan; ⁴Ctr. for Organismal Studies, Univ. of Heidelberg, Heidelberg, Germany; ⁵Mol. and Cell Biol., Univ. of California at Berkeley, Berkeley, CA; ⁶Dept. of Energy Joint Genome Inst., Walnut Creek, CA

Abstract: In vertebrates, protocadherins (PCDHs) are the largest group within the cadherin superfamily, with over 60 members in mouse and human. Genetic studies in mice have demonstrated that PCDHs are critical for neural circuit formation and that their diversity may play a key role in neuronal self-recognition. Within a cell, PCDHs form tetramers without any apparent isoform specificity. Intercellular binding, by contrast, is strictly homophilic (Schreiner and Weiner, 2010). The complexity of assembled extracellular cadherin tetramers allows for hundreds of thousands of specific cell-to-cell interactions. PCDHs are absent from the genome of the protostome model organism D. melanogaster, but the role of PCDHs in vertebrate neural circuit assembly appears analogous to that conferred in flies by Dscam1 alternative splicing diversity. Cephalopods, which have the largest nervous systems among all invertebrates, are capable of extraordinary behaviors including complex problem solving, sophisticated tactile and visual learning, and adaptive coloration. Here, we report that the octopus has the largest number of PCDH genes found in any animal to date. We identified 168 PCDH genes in the genome of Octopus bimaculoides. The octopus complement of PCDH genes resembles vertebrate PCDHs in protein domain architecture. In both lineages, PCDH genes are found in clusters on the genome. Our transcriptome data indicate that PCDH genes are highly enriched in central nervous tissues such as the optic lobe and the supraesophageal brain, which contains higher centers of motor control, decision-making, and learning and memory. Phylogenetic analysis establishes that vertebrates and cephalopods independently evolved a large repertoire of PCDH genes. The extensive similarities between vertebrate and cephalopod PCDH genetic structure provide a striking example of convergent evolution between the two taxa, which are both noted for their large and complex nervous systems.

Poster

473. Role of Adhesion in the Development of Neuronal Wiring

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 473.07/A26

Topic: A.04. Axon and Dendrite Development

Support: NIH Grant MH099453

Title: Differential expression of δ-Protocadherins in identified neurons in zebrafish

Authors: *J. D. JONTES, S. COOPER, M. EMOND; Dept Neurosci, Ohio State Univ., Columbus, OH

Abstract: The patterns of neural connectivity in the mature vertebrate nervous system are highly stereotyped, yet the mechanisms responsible for generating this organization are not known. Differential gene expression defines both regional compartments and distinct neuronal subtypes, but it remains to be shown how this molecular architecture contributes to establishing functional architecture. Among the molecules proposed to contribute to circuit assembly are the δ-protocadherins (δ-Pcdhs), a family of cell surface molecules that are differentially expressed in the developing nervous system. To investigate the involvement of δ-Pcdhs in the assembly of early neural circuits, we have generated recombinant BAC clones and BAC transgenic zebrafish lines for several of these genes. The larval zebrafish is an excellent system in which to address this question, as it is small and relatively simple, yet can mediate a range of complex behaviors. In addition, larvae are transparent and are ideal for live imaging. Using in vivo imaging of both fluorescently labeled transgenic lines and transiently expressing larvae, we have begun to map out the cell types and neural pathways defined by the expression of δ-Pcdhs at multiple stages of development. Neuronal types are identified on the basis of position, morphology and axon trajectory. We find that individual δ-Pcdhs are expressed in stereotyped complements of neurons throughout the nervous system. In addition, we have begun using GCaMP6s to look for patterns of activity in pathways defined by δ-Pcdh expression.

Disclosures: J.D. Jontes: None. S. Cooper: None. M. Emond: None.
473. Role of Adhesion in the Development of Neuronal Wiring

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 473.08/A27

**Topic:** A.04. Axon and Dendrite Development

**Support:** Brain Research Foundation

**Title:** Structural insights into the flrt/latrophilin complex in brain development

**Authors:** *D. ARAC-OZKAN;
Biochem. & Mol. Biol., Univ. of Chicago, Chicago, IL

**Abstract:** FLRTs are cell-adhesion molecules with emerging functions in cortical development and synapse formation. Their extracellular regions interact with Lphns to mediate synapse development, and with Unc5/Netrin receptors to control the migration of neurons in the developing cortex. Here, we present the crystal structures of FLRT3 in isolation and in complex with Lphn3. The FLRT3/Lphn3 structure reveals that Lphn3 binds to FLRT3 at a distinct site from Unc5, but at the same concave surface as the previously reported dimerization surface. Structure-based mutations specifically disrupt FLRT3/Lphn3 binding, but do not disturb their interactions with other proteins or their cell-membrane localization. Thus, they can be used as molecular tools to dissect the functions of FLRTs and Lphns in vivo. Our results suggest that Unc5 and Lphn3 can simultaneously bind to FLRT3 forming a trimeric complex and that FLRT3 may form trans-synaptic complexes with both Lphn3 and Unc5. These findings provide molecular insights for understanding the role of cell adhesion proteins in brain development.

**Disclosures:** D. Arac-Ozkan: None.

Poster

474. Dendritic Growth and Branching

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 474.01/A28

**Topic:** A.04. Axon and Dendrite Development

**Support:** Research Grants Council of Hong Kong (HKUST660810, HKUST661111, HKUST661212 and HKUST660213)
Title: Role of Cdk5 in activity-induced gene expression and dendrite development

Authors: *T. YE*1,2,3, Z. LIANG1,2,3, X. ZHOU1,2,3, K.-O. LAI1,2,3,4, A. K. FU1,2,2, N. Y. IP1,2,3; 1Div. of Life Sci., 2Mol. Neurosci. Ctr., 3State Key Lab. of Mol. Neurosci., The Hong Kong Univ. of Sci. and Technol., Hong Kong, China; 4Dept. of Physiol., Univ. of Hong Kong, Hong Kong, China

Abstract: Proper dendrite growth and arborization in response to sensory experience are essential for neural connectivity and information processing in the brain. Although neuronal activity is important for shaping dendrite morphology, the underlying molecular mechanisms are not well understood. Here, we identified cyclin-dependent kinase 5 (Cdk5)-mediated transcriptional regulation as a key mechanism that controls activity-dependent dendrite development in neurons. Biochemical fractionation and time-lapse imaging demonstrated that Cdk5 became enriched in the nucleus in response to neuronal activity. Knockdown of Cdk5 impaired activity-induced dendritic growth; these defects could be rescued by wild-type Cdk5 but not the nuclear-localization deficient mutant, indicating that the nuclear localization is required for Cdk5 function in dendrite development. Genome-wide analysis revealed that Cdk5 function is mediated through activity-dependent gene expression, including that of brain-derived neurotrophic factor. These findings collectively suggest that the nuclear import of Cdk5 is crucial for activity-dependent dendrite development by regulating gene transcription during nervous system development.


Poster

474. Dendritic Growth and Branching

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 474.02/A29

Topic: A.04. Axon and Dendrite Development

Title: The Purkinje cell forest shapes the trees
Authors: **B. TORBEN-NIELSEN, E. DE SCHUTTER**; Okinawa Inst. of Sci. and Technol., Kunigami-Gun, Japan

**Abstract:** Purkinje neurons are at the heart of all theories about the olivo-cerebellar circuit as they provide the only output from the cerebellar cortex. Hallmark is their planar morphology in a plane perpendicular to the parallel fibers. Moreover, individual Purkinje neurons have very dense dendritic arborizations, a feature commonly referred to as space-filling. In a population, they neatly align and cover the available volume in both transverse and medio-lateral directions, so-called tiling. It remains an open question how individual neurons develop a mono-planar dendrite and how the alignment emerges in a population. Here we address both questions. To address these questions we use our recently developed computational framework, NeuroMaC [1], to simulate the simultaneous growth of large numbers of neuronal morphologies in virtual brain tissues. In order to study planarity and overall alignment of Purkinje neurons, we extended NeuroMaC to allow interstitial (lateral) branching and pruning of branches, which are experimentally demonstrated to play a crucial role in space-filling [2]. In this work, we hypothesized that the peculiar Purkinje neuron morphology emerges from three simple interactions with its environment. First, dendritic branches of one neuron are repelled by each other, a process called self-avoidance. Second, dendritic branches of different neurons repel each other. Third, all branches are to some extent attracted to the pial surface. Simulated somata were place on a regular lattice; 25 and 150 micron apart from each other in, respectively, the medio-lateral and transverse directions. In addition, pruning of branches took place early during simulated growth to match the experimental data [2]. We found that these simple rules yielded highly realistic morphologies exhibiting the hallmark planarity in the transverse direction. The planarity emerged because the repulsion between branches forced the neuron to occupy all available space, which was larger in the transverse than in the medio-lateral direction. We validated the resulting morphologies against exemplar adult data and developmental data and found a good statistical match. Moreover, alignment and tiling emerged in the population. We hence demonstrated that the notoriously complex Purkinje neuron morphology can be approximated by simple local interactions between growth cones. We also speculate on the necessity of a global organization mechanism that consistently aligns the cell bodies of Purkinje neurons in the medio-lateral direction. References: [1] Torben-Nielsen B & De Schutter E, Front. Neuroanat. 8:92 (2014) [2] Fujishima et al., Development 139:3442-3455 (2012)

**Disclosures:** B. Torben-Nielsen: None. E. De Schutter: None.

**Poster**

**474. Dendritic Growth and Branching**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM
Title: E2F1 depletion in neurons results in loss of neurite arborization and changes in synaptic markers during neuron development

Pathology, Univ. of Pennsylvania, Philadelphia, PA

Abstract: E2F1 is a cell cycle transcription factor known for its role in regulating progression from G1 to S phase of the cell cycle. Surprisingly, E2F1 is localized in the cytoplasm of post-mitotic neurons where its role is largely unknown, and where it is unlikely to function as a transcription factor. Aged E2F1 mutant mice show behavioral and synaptic deficits, indicating that E2F1 is important for maintenance of synapses in aging. In addition, E2F1 has been shown to regulate apoptosis and contribute to neurodegeneration. However, its role in the post-mitotic developing neuron has not been described. We show that E2F1 expression increases during neuronal development peaking during synapse formation, indicating that it could play a role in the neuron as neurite outgrowth occurs and synapses form. Using complementary approaches, we have shown that loss of E2F1 results in decreased neurite arborization during a specific developmental period. In addition, loss of E2F1 results in decreased PSD95 and GAP43 levels during development. These data indicate that E2F1 plays an unexpected and novel role in regulating neurite outgrowth during development.

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

Authors: *A. M. GARRETT*1, A. L. TADENEV1, A. KHALIL2, P. G. FUERST3, R. W. BURGESS1;
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Abstract: During development, neurons balance attractive and repulsive signals to properly position cell bodies and neurites, and to form synapses with appropriate partners. The Dscams (from Down syndrome cell adhesion molecule) are Ig-superfamily members important for self-avoidance. *Drosophila Dscam1* promotes self-avoidance by generating up to 19,008 strongly homophilic isoforms, providing a code by which neurites can recognize and actively repel those from the same cell while still interacting with neighboring cells. Mammalian Dscams - *Dscam* and *Dscam1* - serve a similar function without extensive isoform diversity. 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 isoform overlap extensively with other cells expressing the same gene, suggesting that normal homotypic avoidance does not involve a strong repulsive mechanism. Reasoning that this self-avoidance was likely to involve intracellular signaling common to both DSCAM and DSCAML1, we chose to focus on the C-terminal PDZ-interacting domain: The sequences encoding the final ten amino acids of both DSCAM and DSCAML1 were replaced by epitope tag sequences to eliminate the canonical PDZ-interacting domains. These mutations completely recapitulated the null phenotypes in some cell types, but left others relatively unaffected, leading us to hypothesize that Dscams mask cell-type-specific repertoires of CAMs to prevent excessive adhesion, and that the PDZ-interacting domain is required to mask only some of these classes of CAMs. To test this, we focused on retinal ganglion cells labeled in *Cdh3-GFP* transgenic mice, a population that depends on DSCAM for self-avoidance and expresses a known repertoire of CAMs including *Cdh3* and *Cdh6*. We reasoned that if excessive adhesion provided by these Cadherins were normally masked by DSCAM, but were mediating unbalanced adhesion in our *Dscam* mutants, then Cadherin/ Dscam double-mutants would partially rescue the fasciculation phenotype. We quantified fasciculation in these double-mutants and found that indeed there is reduced fasciculation compared to *Dscam* null mice. We are currently testing other candidate adhesion systems in other cell types to ask if making double mutants with Dscam and PDZ-interacting CAMs can rescue fasciculation in cell types that require the PDZ interaction, and if DSCAM can mask multiple classes of adhesion molecules.

Consequences of malformed versus signaling-depleted neuronal cilia during cortical development

Authors: A. PARKER¹, M. LE¹, J. COLEMAN², *M. R. SARKISIAN¹;
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Abstract: Nearly all cortical neurons grow and maintain a primary cilium, but how signaling from these structures shape brain development is poorly understood. Our previous published work shows that malformation of cilia or loss of type 3 adenylyl cyclase III (ACIII) signaling from cilia during development results in the failure of cortical pyramidal neurons to properly elaborate dendritic arbors. Thus, we hypothesized that neurodevelopmental insults which lead to long-term changes in brain circuitry and function, such as early life seizures, would alter the developmental trajectory of neuronal cilia. By immunostaining for cilia markers, our recent data suggest that neuronal, but not glial ciliogenesis, is significantly disrupted by pentylenetetrazole-induced seizures during early neonatal development, leading to both acute and long-term changes in the length of cilia. Using a modified rabies virus to transsynaptically label neurons within seized brains with GFP, our preliminary data suggest the seizure-induced ciliary changes overlap with a reduction in cortical neuronal connectivity. These observations have led us to ask whether changes in neuronal cilia structure affect dendritic development in a manner similar to changes in ciliary signaling. We are currently using in utero electroporation at E15.5 to deliver and compare the effects of CRISPR/Cas9 plasmids targeting Kif3a (to abrogate ciliogenesis) or ACIII (to disrupt cilia signaling) in specific postnatal developing neocortical neurons. This approach will allow us to test the hypothesis that a loss/reduction of ACIII from a neuronal cilium also impairs dendritic development and synaptic connectivity. The combined use of neuroanatomical, morphological and calcium imaging techniques to characterize subsets of neurons lacking key cilia signaling molecules will help to further define the importance of cilia in neuronal development and provide deeper insight into how common neurodevelopmental insults may alter the course of brain development and function.

Poster

474. Dendritic Growth and Branching

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 474.06/A33

Topic: A.04. Axon and Dendrite Development

Support: NIH Grant NS055272

Title: Dendrite arborization in cortical neurons depends on $\gamma$-Protocadherin-mediated homophilic matching with surrounding neurons and astrocytes

Authors: *M. J. MOLUMBY, A. B. KEELER, J. A. WEINER;
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Abstract: The $\alpha$-, $\beta$-, and $\gamma$-Protocadherins ($\gamma$-Pcdhs) are cadherin superfamily adhesion molecules encoded by clustered gene families. The 22 $\gamma$-Pcdhs are combinatorially expressed in the CNS by neurons and astrocytes, and play critical roles in synaptogenesis, dendrite arborization, and the survival of subsets of neurons. We showed previously that the $\gamma$-Pcdhs promiscuously form cis-multimers that interact strictly homophilically in trans (Schreiner and Weiner, PNAS, 2010); the $\alpha$- and $\beta$-Pcdhs were subsequently shown to interact in a similar homophilic manner. The Pcdh gene clusters thus have the potential to generate millions of distinct adhesive interfaces, providing CNS cells with molecular identities. We demonstrated previously that, in mice lacking the $\gamma$-Pcdhs in the cerebral cortex, pyramidal neurons exhibit severely reduced dendrite arborization (Garrett, et al., Neuron, 2012). This, combined with many studies of $\gamma$-Pcdh interactions \textit{in vitro}, suggests that homophilic, adhesive $\gamma$-Pcdh interactions between neurons, and between neurons and glia, provide a positive signal for dendrite growth. However, in retinal starmburst amacrine cells and cerebellar Purkinje cells, loss of the $\gamma$-Pcdhs resulted in aberrant dendrite fasciculation and self-crossing, suggesting that these molecules can mediate repulsive self-avoidance between a neuron’s own dendrites. Here we provide multiple lines of evidence that homophilic matching of $\gamma$-Pcdhs in trans, between cells, is critical for proper formation of cortical neuron dendrite arborrs. We demonstrate that expressing a single $\gamma$-Pcdh isoform in all primary neurons and astrocytes of the cortex \textit{in vivo}, such that all cells “match” homophilically, greatly increases dendrite arborization compared to control mice. Additionally, astrocyte-specific loss of the $\gamma$-Pcdhs cell-non-autonomously decreases dendrite arborization in neurons, even though they retain their normal $\gamma$-Pcdh complement. Conversely, driving expression of a single $\gamma$-Pcdh isoform in an isolated cortical neuron, surrounded by wild-
type cells, decreases arborization in the transgenic neuron. Experiments in which cortical neurons expressing a particular γ-Pcdh isoform are co-cultured with other cortical neurons expressing the same, or a different γ-Pcdh isoform, support these \textit{in vivo} data: dendrite arborization is greatest when a neuron is surrounded by homophilically matching cells. We conclude that, in cortical neurons, the γ-Pcdhs regulate dendrite arbor growth and morphology not through repulsive self-avoidance, but through positive homophilic trans-interactions between cells.

**Disclosures:** M.J. Molumby: None. A.B. Keeler: None. J.A. Weiner: None.

**Poster**

**474. Dendritic Growth and Branching**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

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**Topic:** A.04. Axon and Dendrite Development

**Support:** NIH Grant NS083378-12

**Title:** The trafficking and localization of neuron-enriched endosomal protein (Neep21) in neurons

**Authors:** *C. YAP, L. C. DIGILIO, L. P. MCMAHON, B. WINCKLER; Neurosci., Univ. of Virginia Sch. of Med., Charlottesville, VA

**Abstract:** Neuronal endosomes are essential for membrane receptor trafficking in neurons. Endosomes participate in a variety of signaling events, as well as regulating the rates of recycling and degradation. Endosomal trafficking is thus at the center of receptor trafficking in neurons and participates in various neuronal functions, such as synaptic plasticity and axon outgrowth. Dysfunctions of the endolysosomal system have been implicated in a number of neurodegenerative conditions. As in other cell types, neuronal endosomes are regulated by a variety of endosomal regulators, which localize to different endosomal compartments. Neep21 (neuron-enriched endosomal protein of 21 kD) is a neuronal-specific endosomal transmembrane protein, localized to Golgi and endosomal compartments in the somatodendritic domain, and is important for trafficking of L1/NgCAM, AMPAR, and processing of beta-amyloid. In this work, we characterized the localization of Neep21 in detail and studied its trafficking and dynamic relationships with endosomal regulators rab5 and rab7 in neurons. We found that Neep21 co-localized with EEA1 and rab5, as expected from previous work. Neep21 also colocalized with Rab7 in the soma and along dendrites, but rarely colocalized with Lamp1, a lysosomal marker.
Importantly, we discovered that localization of Neep21 is regulated by Rab5 and Rab7. Overexpression of constitutively active Rab5CA (Rab5Q79L) retained Neep21 in enlarged early endosomes, whereas overexpression of Rab5 dominant negative (Rab5S34N) caused diffuse distribution of Neep21 along the plasma membrane of the soma and dendrites, and less vesicular-form of Neep21. Most importantly, we found that Rab5DN caused retention of Neep21 on the plasma membrane, and blocked endocytosis of Neep21 from the plasma membrane. In addition, endocytic trafficking of Neep21 from early endosomes is regulated by Rab7. Downregulation of Rab7 expression (by shRab7) and overexpression of Rab7 dominant negative (Rab7T22N) induced formation and accumulation of enlarged Neep21 early endosomes, indicating trafficking of Neep21 from early endosome requires Rab7. Interestingly, we found that Neep21 is also sorted to late endosomes for degradation as treatment with leupeptin, a lysosomal proteases inhibitor, induced accumulation of elongated- and enlarged-Neep21 endosomes in the soma and along dendrites, and treatment with protein synthesis inhibitor, cycloheximide, depleted the protein level of neep21 in neurons. This indicates that localization of Neep21 is not limited only to early endosomal compartment, it is also localized and trafficked toward late endosome/lysosome for its own degradation.


Poster

474. Dendritic Growth and Branching

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 474.08/A35

Topic: A.04. Axon and Dendrite Development

Support: NSF IGERT 0965918

NSF STC EBICS CBET 0939511

NSF CBET 1040462

NSF EAGER DBI 1450962

NIH R21 MH101655

Title: High-resolution analysis of semaphorin3A effects on the dynamics of filopodia on the tips and shafts of developing dendrites using SLIM imaging


**Authors:** *A. JAIN*¹, T. KIM², G. POPESCU², M. U. GILLETTE¹;
¹Dept. of Cell and Develop. Biol., ²Electrical & 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. 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 growth and shrinkage rates. This is made possible through Spatial Light Interference Microscopy (SLIM), an innovative quantitative phase imaging method for high-resolution, label-free imaging of live cells through interferometry (Wang et al., Opt. Express, 2011). SLIM permits measurement 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**

**474. Dendritic Growth and Branching**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 474.09/A36

**Topic:** A.04. Axon and Dendrite Development

**Title:** Mechanisms of dendritic trafficking and microtubule dynamic regulation

**Authors:** *A. E. GHIRETTI*, E. L. F. HOLZBAUR;
Univ. of Pennsylvania, Philadelphia, PA
Abstract: Neurons are highly polarized cells, extending distinct processes specialized to send (axons) and receive (dendrites) information. The accurate trafficking of the correct protein cargoes to either the axonal or dendritic compartments is required to maintain this polarity and therefore proper neuronal function. This process is regulated by dynein and kinesin motor proteins that move along the microtubule cytoskeleton. Importantly, the mislocalization of protein cargos or dysfunction of microtubule and motor proteins is implicated in a number of neurological disorders, including neurodevelopmental disorders such as mental retardation and autism. While axonal trafficking is well studied due to the uniform polarity of axonal microtubules, dendritic microtubule structure is more complex; thus, the motors that mediate dendritic trafficking as well as the dynamics of dendritic microtubules themselves remain largely unknown. We have utilized single molecule assays and live imaging in rodent hippocampal neurons to more fully characterize the function of kinesin in dendrites. Ultimately, our studies will help us to understand how the full repertoire of dendritic kinesins function to promote the functional specification of the dendritic and axonal compartments, and shed light on the little studied but essential process of dendritic trafficking.

Disclosures: A.E. Ghiretti: None. E.L.F. Holzbaur: None.

Poster

474. Dendritic Growth and Branching

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 474.10/A37

Topic: A.04. Axon and Dendrite Development

Support: Deutsche Forschungsgemeinschaft (SFB 1089)

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    Else Kröner-Fresenius-Stiftung

    BONFOR program of the University of Bonn Medical Center.

Title: Ste20-like kinase is a regulator of dendritic arborization and excitation-inhibition balance in neocortical neurons

Authors: *B. K. ROBENS¹, R. MARESCH², A. GROTE³, K. M. J. VAN LOO¹, H. BECK², S. SCHOCH¹, A. BECKER¹;
Abstract: Dendrite arborization and subsequent proper formation and maintenance of inhibitory and excitatory synapses are critical for the emergence of functional adult neuronal networks. Key regulators in forming and stabilizing dendritic arbor with their precise spatial arrangement of inhibitory vs. excitatory synapses have yet to be discovered. Here, we show that the Ste20-like kinase (SLK) has a key regulatory role in these processes. So far, SLK has been shown to be important for migration of fibroblasts by remodeling the cytoskeleton and for cell cycle progression. We show that shRNA mediated silencing of murine SLK or overexpression of the kinase dead (KD) SLK causes a striking and selective loss of distal dendritic arbors in neuronal cultures. Intraventricular in utero electroporation (IUE) of SLK shRNAs resulted in a similar cellular phenotype in cortical neurons, and additionally revealed a migration deficit of affected neurons. Surprisingly, the loss of distal, mainly excitatory input sites was associated with significantly elevated cortical excitability in vivo. A closer functional examination revealed a profound deficit in inhibition in affected neurons. Inhibitory synapse density and inhibitory transmission was progressively reduced at ages > P15. In contrast, excitatory synapses and neurotransmission were unchanged. Interestingly, neurons from human ganglioglioma tissue exhibited reduced SLK immunoreactivity in comparison to adjacent pre-existing control neurons, linking reduced SLK protein levels to an epilepsy-associated neuronal disorder. These data indicate a critical role for SLK during development particularly for dendritic arbor formation as well as the proper balance of dendritic inhibitory and excitatory synapses. They also suggest a role for reduced SLK protein expression in mediating functional changes and hyperexcitability in patients with ganglioglioma.


Poster

474. Dendritic Growth and Branching

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

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Topic: A.04. Axon and Dendrite Development

Support: PICT 2010-1012 grant (FONCyT, ANPCyT)

PICT 2013-0914 grant (FONCyT, ANPCyT)
Title: The leucine-rich repeat transmembrane protein Lrig1 regulates dendrite arborization and social interaction in mice

Authors: *F. C. ALSINA, F. J. HITA, P. FONTANET, D. IRALA, F. LEDDA, G. PARATCHA;
Inst. of Cell. Biol. and Neurosci., Buenos Aires City, Argentina

Abstract: Dendrite size and morphology are key determinants for the functional properties of neurons and numerous neurodevelopmental and psychiatric disorders are mainly due to structural abnormalities of dendrites and their connections. Dendritic tree development is believed to result from the interaction between extracellular signals (such as neurotrophic growth factors), intrinsic modulators and electrical activity. Compared with the many identified factors that promote general dendritic growth and branching, little is known about the cell-type specific modulators that allow neurons to sculpt distinctive dendrite patterns. In the current study we show that Lrig1 - a pan-receptor tyrosine kinase associated protein that modulates many growth factor receptor signaling - is a physiological inhibitor of hippocampal dendrite morphogenesis and branching. Lrig1-deficient hippocampal neurons display an enhanced primary dendrite formation and proximal dendritic branching. Interestingly, Lrig1 KO mice exhibit enhanced proximal dendritic arborization of hippocampal pyramidal neurons, low sociability and social interaction deficits. Taken together, our findings reveal an unexpected role of Lrig1 in the control of hippocampal dendrite development and plasticity, probably through the inhibition of neurotrophic growth factor-induced signaling.


Poster

474. Dendritic Growth and Branching

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

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Topic: A.04. Axon and Dendrite Development

Support: NIH EY024694

E. Matilda Ziegler Foundation
Title: Assembly of the retinal direction-selective circuit is coordinated by starburst amacrine cells

Authors: *T. RAY, M. STOGSDILL, J. KAY; Duke Univ., Durham, NC

Abstract: During retinal development neurons form parallel circuits tuned to convey specific features of the visual scene. At the inner plexiform layer (IPL) of the retina, neurons send their axons and dendrites to specific IPL sublayers where they form circuit-specific synaptic connections. The mechanisms employed by neurons to achieve such specificity are not well understood, but are of great biological importance because these processes are fundamental to sensory perception. To determine the mechanisms neurons use to form cell type specific connections we use the mouse direction-selective (DS) circuit as a model. Major components of the DS circuit include starburst amacrine cells, type 5/7 bipolar cells, and direction-selective ganglion cells (DSGCs) that coalesce at specific IPL sublayers to form synaptic connections. Here we test the hypothesis that starburst amacrine cells organize the formation of the DS circuit through homotypic repulsion and by expressing instructive cues for DS circuit cells. Starbursts stratify within the IPL before other DS circuit neurons placing them in prime position to organize the formation of the DS circuit. To determine how starbursts organize the DS circuit we devised a genetic strategy to label starbursts during early development and to perturb their dendritic patterning in the IPL. The latter was achieved by knocking out a gene, *Megf10*, that mediates homotypic recognition among starbursts. In the *Megf10* mutant, starbursts exhibit sporadic IPL errors including clumps and gaps in IPL innervation in addition to ectopic IPL layers. Disruption of starburst IPL stratification allows us to ask two key questions about the formation of the DS circuit: 1) Is homotypic repulsion needed for setting up the initial starburst IPL layers? 2) Do starbursts express cues that guide DS circuit neurons to the proper IPL location? We found that starburst IPL stratification is delayed in *Megf10*−/− mutants. Further, starbursts are instructive in recruiting DSGCs and type 5/7 bipolar cells to the appropriate IPL sublayer. DSGC IPL projections mirror the misprojections of starbursts resulting in DSGC avoidance of IPL regions devoid of starbursts and innervation of ectopic starburst IPL layers, indicating starbursts attract the DSGCs. However, we found that type 5/7 bipolar cells can still project to IPL regions without starburst arbors. Additionally, we found that starbursts express repulsive cues for type 5/7 bipolar cells that target them to their precise IPL positions. These results demonstrate that starbursts actively guide DS circuit neurons, including other starbursts, to their appropriate IPL position by expressing guidance cues.

Title: Dendritic remodeling by the Angelman syndrome and autism protein E6AP

Abstract: Following growth and extensive branching of dendrites, structural maturation of neurons is characterized by a pruning process in which the extra, non-functional dendritic branches are actively removed. The timing of pruning and selection of specific dendrites determines the wiring of neural circuits and, ultimately, brain function. Angelman Syndrome (AS) and some autism spectrum disorders (ASDs) are caused by genetic disruption of E6AP, an E3 ligase that targets multiple proteins for ubiquitination and proteasome-mediated degradation. However, the neuronal function of E6AP remains largely unknown. We find that overexpression of E6AP leads to a reduction in dendritic arborization via dendritic pruning. We show that E6AP causes activation of the caspase proteases, a pathway known for local neurite degeneration and spine pruning. E6AP targets an endogenous caspase inhibitor for ubiquitination and degradation, therefore removing the inhibition of caspases and inducing the pruning process. Furthermore, we provide live imaging data suggesting that pruning occurs by distal fragmenting and shrinking of dendrites, eventually leading to the removal of entire dendritic segments. These findings provide novel mechanistic insight into our understanding of the physiological function of E6AP and the pathogenesis of Angelman syndrome and ASDs. Additionally, this study provides the first in vitro model of dendritic pruning in mammalian neurons.

Disclosures: N. Khatri: None. J. Gilbert: None. M. Nee: None. H. Man: None.
**Title:** Role of ryanodine receptors in rat subventricular zone development

**Authors:** *B. A. DOS SANTOS*, M. V. DAMICO, E. R. KINJO, G. S. HIGA, F. A. DOS SANTOS, A. H. KIHARA;

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2Dept. de Biofísica e Fisiologia, Inst. de Ciências Biomédicas, Univ. de São Paulo, São Paulo, Brazil

**Abstract:** During development of the central nervous system (CNS), the formation of the olfactory bulb occurs through the proliferation and migration of postnatal differentiated neurons from the subventricular zone (SVZ) close to the lateral ventricle. The activity of SVZ cells is modulated by the variation of intracellular calcium concentration, which in turn depends on external calcium and intracellular stores, localized in organelles such as mitochondria and endoplasmic reticulum. These organelles accumulate ryanodine receptors (RyRs 1, 2 and 3) that control the release of calcium into the cytosol. The aim of this project was to verify the role of these receptors and, consequently, the participation of intracellular calcium in the development of the SVZ cells. To this end, we first analyzed gene expression through the real time PCR technique of these receptors and also descriptive analysis related to the protein distribution by immunofluorescence in the SVZ of neonate animals in the day of birth (P0). We also evaluated Ki-67 colocalization with RyRs in order to analyze its accumulation in active phases of the cell cycle. Then we performed *in vitro* blockade of RyRs in SVZ cells, isolated from P0 animals with dantrolene (5 µM) during 4 hours. After the blockade, possible alterations were evaluated i) cell morphology; ii) neurite growth. For this, combined techniques such as immunohistochemistry and real time PCR were used, besides morphological evaluations. We observed lower gene expression of the three isoforms of RyRs in neonates compared to adults. Immunofluorescence analysis revealed that the three RyRs isoforms are similarly distributed throughout the SVZ of P0 animals. Besides, all isoforms accumulate in Ki-67-positive cells in neonates, demonstrating the presence of RyRs during the cell cycle. *In vitro* blockade of RyRs in primary cell culture from SVZ inhibited neurite growth, as well as avoided the progress of the circular morphology of the cells to a differentiated shape with increased dendritic branching, indicating that RyRs participate in important cellular maturation processes. Thus, we are able to disclose that RyRs...
participate in the development of the cells from SVZ, which migrate and participate in the olfactory bulb circuitries.


Poster

474. Dendritic Growth and Branching

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 474.15/A42

Topic: A.04. Axon and Dendrite Development

Support: NSF Grant IOS1021860

NIH Grant NS073584

NIH Grant 8P30GM103507

Title: Requirement of neuronal ribosome synthesis for growth and maintenance of the dendritic tree

Authors: *M. HETMAN, L. SLOMNICKI, M. PIETRZAK, A. VASHISHTA;
Neurolog. Surgery, Univ. of Louisville, Louisville, KY

Abstract: The nucleolus serves as a principal site of ribosome biogenesis but is also implicated in various non-ribosomal functions including negative regulation of the pro-apoptotic transcription factor p53. While disruption of the nucleolus may trigger the p53-dependent neuronal death, neurotoxic consequences of a selective impairment of ribosome production are unclear. Here we report that in rat forebrain, neuronal maturation is associated with a remarkable expansion of ribosomes despite postnatal downregulation of ribosomal biogenesis. In cultured rat hippocampal neurons, inhibition of the latter process by knockdowns of ribosomal proteins S6, S14 or L4 reduced ribosome content without disrupting nucleolar integrity, cell survival and signaling responses to the neurotrophin BDNF. Moreover, RNA stress granules were formed suggesting diminished ribosome recruitment to mRNAs. Such a translational insufficiency was accompanied by impairment of BDNF-mediated dendritic growth. RNA stress granules and smaller dendritic trees were also observed when ribosomal proteins were depleted from neurons with established dendrites. Thus, a robust ribosomal apparatus is required to carry out protein synthesis that supports dendritic growth and maintenance. Consequently, deficits of ribosomal biogenesis may disturb neurodevelopment by reducing neuronal connectivity. Finally, as stress
granule formation and dendritic loss occur early in neurodegenerative diseases, disrupted homeostasis of ribosomes may initiate and/or amplify neurodegeneration-associated disconnection of neuronal circuitries.

Disclosures: M. Hetman: None. L. Slomnicki: None. M. Pietrzak: None. A. Vashishta: None.

Poster

474. Dendritic Growth and Branching

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Topic: A.04. Axon and Dendrite Development

Support: NIH R21 MH101655
NSF STC EBICS CBET 0939511
NSF CBET 1040462
NSF BRAIN EAGER DBI 1450962

Title: Keeping filopodia dynamic: exploring the role of miR-125b in dendrites using real time imaging

Authors: *R. IYER*¹,², T. KIM³,², G. POPESCU³,², M. U. GILLETTE¹,²;
¹Cell and Developmental Biol., ²Beckman Inst., ³Electrical & Computer Engin., ⁴Neurosci. Program, Univ. of Illinois At Urbana-Champaign, Urbana, IL

Abstract: Developing dendrites encounter a multitude of stimuli that direct their growth and architecture. Cellular substrates respond to these stimuli, integrating extrinsic information to direct dendritic development. Of emerging interest in this process are microRNAs, small noncoding RNAs ~22 nucleotides long that can reversibly repress local translation. By responding to external cues sensed by dendritic filopodia, they participate in key decision-making processes in developing dendrites: where and how to grow and mature. We are focusing on miR-125b, a brain-abundant microRNA, to elucidate its role in the dynamics of filopodia and the corresponding changes in filopodial and dendritic structure. We inhibit miR-125b’s activity in cultured hippocampal neurons during the early stages of development (4-7 DIV) as filopodia explore their microenvironment. Selective inhibition of miR-125b increases the width of filopodia, and negatively impacts dendrite development. To understand the effect of miR125b on
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 show that the rate of filopodial extension and retraction is reduced significantly when miR125b is inhibited. We propose that miR-125b is critical in maintaining the filopodial phenotype early in dendrite development, and in laying the complex dendrite arbor. These high-resolution analyses reveal fresh insights into the process by which neurons integrate multiple external signals to establish the correct connections. Such insights are critical to understanding roles of miR125b in neurological disorders such as Fragile X Syndrome and Alzheimer’s disease.


Poster

474. Dendritic Growth and Branching

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 474.17/A44

Topic: A.04. Axon and Dendrite Development

Support: Lycoming College Haberberger Fellowship to TF

Title: Purkinje neuron axonal and dendritic markers during development in vivo and in vitro

Authors: T. FAULL, J. N. BATTYANYI, 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 over 200,000 granule cell parallel fibers and climbing fibers originating from the inferior olivary nuclei. 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. Here we study 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? Previous studies suggested that some individual early processes might express protein markers for both axons and dendrites. Interpretation of these results was limited by 2-color imaging, making it difficult to prove that these processes were indeed derived from Purkinje neurons. In the current study, 3-color immunohistochemistry of cryostat sections and immunocytochemistry of cultured cerebellar cells will be 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. Faull: None. J.N. Battanyi: None. L.M. Robertson: None. A.R. Demchak: None. M.E. Morrison: None.

**Poster**

474. Dendritic Growth and Branching

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 474.18/A45

**Topic:** A.04. Axon and Dendrite Development

**Support:** NIH Grant NS073919

**Title:** *In vivo* imaging of Cerebellar Granule cell dendritic arborization and synaptogenesis

**Authors:** *M. DHAR*¹, A. W. HANTMAN², N. NISHIYAMA¹, H. NISHIYAMA¹; ¹Neurosci., Univ. of Texas At Austin, Austin, TX; ²Howard Hughes Med. Inst., Ashburn, VA

**Abstract:** Appropriate dendritic arborization and synapse formation is vital for proper circuit formation. Numerous *in vitro* studies have elaborated on this process, but the *in vivo* environment is considerably more complex. Therefore, we are interested in studying this developmental process *in vivo* using chronic time-lapse imaging. We used cerebellar granule neurons (CGNs) as our model neuron since they undergo dendritic arborization and synaptogenesis post-birth from post-natal day 10-21 (P10-21). Using a new transgenic mouse line that sparsely labels only CGNs in the cerebellar cortex, we were able to characterize the dendritic and synaptic development of these neurons *in vivo*. A cranial window (approx. 1mm²)
was created on top of the cerebellar vermis (lobule 6/7) of neonatal mice (P6-7). Three days post-surgery, immature CGNs within the cranial window were imaged every day from P11-P23. A total of 9 CGNs from 4 animals were imaged; 6 were within 150µm of the brain surface while 3 were deeper than 200µm. All the cells had excess numbers of dendrites at the earliest time point (P12, 6 ±1 total dendrites) indicating immature CGN morphology. However, within three days (P15) all CGNs imaged had undergone dendritic pruning such that the remaining dendrites were the final surviving dendrites and no further additions or deletions from the dendritic arbor occurred (P23, 4.2 ±0.7 total dendrites). Concurrent with the decrease in dendritic number, the total dendritic length also reduced from P12 to P15 (P12: 99.89 ±0.97µm, P15: 74.34 ±8.17µm, p=0.03). However, after the establishment of final surviving dendrites at P15, dendritic length steadily increased but only until P18, reaching a maxima that was maintained from then on (P18: 90.57 ±5.79µm, P23: 91.08 ±9.01µm, p=0.48). Mature CGN dendrites end in a claw-like structure which is the site for both excitatory and inhibitory synapses. We observed claw formation in the maturing CGNs and found that all the immature CGNs developed a claw-like ending on at least one of their dendrites between P13-P15 with shallower CGNs developing earlier (at P13) while all the deeper CGNs developing later (at P15). This delay in development was also evident when comparing the time points the CGNs had a claw-like ending on all their remaining dendrites. Deeper CGNs reached this stage at P19 while shallower CGNs were morphologically mature by P17-18. Since no dendrite with a claw was retracted, it suggests an interplay between the processes of synaptogenesis and dendritic development. Hence, we are interested in the role of the claw and the pre-synaptic structures that synapse on to the claw in the development of CGN dendritic arbor.

Disclosures:  M. Dhar: None. A.W. Hantman: None. N. Nishiyama: None. H. Nishiyama: None.

Poster

474. Dendritic Growth and Branching

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 474.19/A46

Topic: A.04. Axon and Dendrite Development

Support: The INOUE ENRYO Memorial Foundation for Promoting Sciences

Title: Age-related changes of collapsin response mediator protein 4 (crmp4)

Authors: *T. KAWACHI1, A. TSUTIYA1, H. MOTEGI1, T. OKADA1, R. OHTANI-KANEKO1,2;
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Abstract: Collapsin Response Mediator Protein (CRMP) was identified as the molecule required for the signal transduction related to the growth cone collapse of axons (Goshima et al., 1995). CRMPs are now known to consist of five homologous cytosolic proteins, CRMP1–5. It has been reported that all of them are highly expressed in the developing as well as adult brain. However, recent our in situ hybridization study revealed that signals of Crmp4 mRNA were occasionally detected in adults, though it was abundantly expressed during early postnatal period (Tsutiya & Ohtani-Kaneko, 2012). Our study also demonstrated that CRMP4 plays an important regulatory role in the growth of not only axons but also dendrites, suggesting its crucial function in building neural networks (submitted). On the other hand, it has been reported that the function of CRMP4 is controlled by phosphorylation of the molecule (Alabed et al., 2010; Fujisawa et al., 2011). However, which phosphorylation sites of CRMP4 are critical for neural developing remains unclear. Therefore, this study aimed to identify the amino acid residue of CRMP4 important for neural development. For the purpose, two dimensional gel electrophoresis (2DE) was used to examine expression changes of different variations of CRMP4 during postnatal development of the mouse brain. Then we identified the phosphorylated residues with UPLC/MS/MS (SYNAPT G2, Waters) spectrometry analysis, whose expression was significantly decreased after postnatal day 7. We then studied the role of these phosphorylation sites of the molecule in vitro. Proteins in the cerebral cortex tissue from male postnatal day 0 (PD0), PD7, 8-week-old (Adult), and over 1-year-old (Aged) mice were subjected to 1DE and 2DE immunoblotting with antibodies against CRMP4 and CRMP4 phosphorylated at Thr 509 (pCRMP4). One DE immunoblotting showed that expressions of CRMP4 and pCRMP4 were significantly decreased in adult and aged mice, compared to those at PD0. Two DE immunoblotting revealed nine spots (No. 1 ~ 9) of CRMP4. All nine spots were dramatically decreased in adult brain, compared to those at PD0. Especially, expressions of 2 spots were rapidly decreased during postnatal period and significantly decreased at PD7, compared to those at PD0. Next, we identified some of phosphorylated residues of these 2 spots. Using mouse hippocampal cells (HT22 cell), we examined the effect of point mutations of these phosphorylation sites on neurite elongation and survival rate of cells.

Title: The mevalonate pathway in the development and survival of mouse Purkinje cells in culture

Authors: *A. BARSZCZYK*¹, H.-S. SUN², Y. QUAN¹, W. ZHENG³, M. P. CHARLTON¹, Z.-P. FENG¹;
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Abstract: Purkinje cells comprise an essential part of the cerebellar circuit, which underlies motor learning and some higher cognitive functions. The mevalonate pathway has important roles in cell survival and development. High cholesterol turnover is associated with dendritogenesis of Purkinje cells during postnatal development. In this study, we compared the effects of mevalonate pathway inhibition on mature and immature Purkinje cells in mouse cerebellar culture. We found that mevalonate pathway inhibition by mevastatin caused a loss of Purkinje cells. GGPP supplementation significantly attenuated this cell death, suggesting that GGPP is required for Purkinje cell survival. Selective inhibition of the squalene-cholesterol branch of the pathway resulted in developmental deficits, suggesting a crucial role for the squalene-cholesterol branch of the mevalonate pathway in the maturation of Purkinje cells. Given that abnormalities in Purkinje cells are associated with motor-behavioral learning disorders such as cerebellar ataxia, caution should be exercised with drugs that inhibit geranylgeranylation or the squalene-cholesterol branch of the pathway in the brain during development.

Disclosures: A. Barszczyk: None. H. Sun: None. Y. Quan: None. W. Zheng: None. M.P. Charlton: None. Z. Feng: None.
Title: ADD3 and KANK1: roles in dendrite morphology

Authors: *J. BRUDVIG*\(^1,2\), N. SAHIR\(^2\), J. CAIN\(^2\), J. WEIMER\(^2\), M. KRUER\(^2\);
\(^2\)Children's Hlth. Res. Ctr., \(^1\)Sanford Res., Sioux Falls, SD

Abstract: KANK1 and ADD3 are cytoskeleton-modulating proteins expressed in neurons. These proteins cap growth at the dynamic (+)-end of actin filaments, and have been shown to influence diverse neuronal processes including vesicle recycling and dendritic spine remodeling. Mutations in *KANK1* and *ADD3* have been associated with heritable forms of cerebral palsy, although the underlying etiology has not been well characterized. shRNA-mediated knockdown of these genes resulted in statistically significant reductions in dendritic complexity in mouse primary neuron cultures as measured by Scholl analysis. Future work will examine the effects of knockdown on dendritic spine density and morphology in cultured neurons, as well as the in vivo effects on dendritic spines and arbors following in utero electroporations with shRNA-encoding plasmids.

Support: NSF Grant IOS-1353724

NJCBI R Grant CBIR14IRG019

Title: Effects of small peptides on protein interactions and dendrite branching

Authors: *H. MENON*¹, H. CHAPMAN², M. SPALLER², B. FIRESTEIN¹;
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Abstract: Dendritic branching is essential for proper development of the neuronal circuitry. Interestingly, a number of neuropsychiatric diseases, such as autism, schizophrenia and Alzheimer’s disease, display abnormal dendritic branching. We previously reported that the protein cypin (cytosolic PSD-95 interactor) is a key player in the process of dendritic branching. The interaction between cypin and PSD-95, via cypin binding to the first two PDZ domains of PSD-95, is essential for the promotion of stable dendrite branches. Cypin is a positive regulator of dendrite branching while PSD-95 inhibits dendrite branching. To test the importance of binding partners to the PDZ domains of PSD-95 in the regulation of dendrite branching, we are characterizing a set of small peptides that specifically bind to the PDZ domains of PSD-95. Our preliminary results indicate that a subset of the compounds alters the interaction between PSD-95 and cypin but possibly not between PSD-95 and another interacting molecule, neuronal nitric oxide synthase (nNOS). Our ongoing work will address whether the small peptides have an effect on dendritogenesis. These studies will allow us to understand how the dendritic tree is shaped and elucidate potential therapies for disorders that show aberrant dendrites.


Poster

474. Dendritic Growth and Branching

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 474.23/A50

Topic: A.04. Axon and Dendrite Development

Support: NIMH Grant 5F30MH096457-02

Title: Regulation of cortical gabaergic interneuron function by the mental disorder susceptibility molecule cntnap2
Authors: *R. GAO, G. ZHANG, D. M. D. SAAVEDRA, P. PENZES;
Physiol., Northwestern Univ., Chicago, IL

Abstract: While complex neuropsychiatric disorders have equally complicated genetic etiologies, recent research revealed common disruptions of individual genes across multiple diseases, suggesting similar pathological mechanisms. For example, several genetic studies have established contactin-associated protein-like 2 (CNTNAP2) as a risk gene in mental disorders such as autism spectrum disorders, schizophrenia, epilepsy, and intellectual disability. Studying the function of CNTNAP2, therefore, can lead to unprecedented insight into shared susceptibility pathways of mental diseases. CNTNAP2 is a member of the neurexin superfamily, is highly expressed in the brain, and clusters potassium channels in the axon’s juxtaparanodes. However, its function outside of the axon is less explored. Recent studies of CNTNAP2 knock-out mice revealed not only behavioral abnormalities and epileptic seizures, but also reductions of cortical interneurons and abnormal neuronal synchrony (Peñagarikano et al., 2011). In addition, CNTNAP2 knockdown in primary neuronal culture resulted in the reduction of both excitatory and inhibitory transmission (Anderson et al., 2012). Taken together, these data implicate CNTNAP2 in controlling E/I balance and behavior, possibly through cortical interneuron function. However, mechanistic studies verifying this hypothesis have not yet been established. To this end, we used a wide array of techniques including yeast-2-hybrid screening, confocal and high resolution imaging, biochemistry, and primary neuronal culture to dissect CNTNAP2’s role in cortical interneuron function. Our data provide novel insight into the molecule’s cellular mechanisms and provide clues to how its dysfunction may contribute to various mental disease etiologies.


Poster

474. Dendritic Growth and Branching

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 474.24/A51

Topic: A.04. Axon and Dendrite Development

Title: The X-linked intellectual disability protein KIAA2022 in neuron morphogenesis and synapse formation

Authors: *J. P. GILBERT1, H.-Y. MAN2;
1Biol., 2Biology, Pharmacol. and Exptl. Therapeut., Boston Univ., Boston, MA
Abstract: Individual neurons must undergo processes of extensive morphogenesis and synapse formation to ensure proper neuronal connectivity and network function. Previous studies of autism spectrum disorders (ASD) have shown abnormalities in brain development, including axon and dendritic outgrowth, synapse formation and plasticity. Our earlier work has identified that loss of function of an X-linked gene, KIAA2022, was the etiological factor in a particular group of patients with intellectual disability and ASD phenotypes. KIAA2022 is a novel protein that lacks known molecular motifs, has no significant homology to other proteins and its neuronal function remains unknown. Using rat hippocampal neurons, we report that KIAA2022 shows a typical nuclear localization, indicating a role for KIAA2022 in gene regulation. We find that knockdown of KIAA2022 results in a marked impairment of neurite outgrowth, synapse formation and synaptic activity. Knockdown of KIAA2022 leads to an increase in total and synaptic N-cadherin levels, as well as an increased association between N-cadherin and δ-catenin. These results indicate that increased N-cadherin levels may sequester δ-catenin at the membrane, thereby preventing its downstream interaction with effectors of neurite morphogenesis and synapse formation. Our findings establish important roles for KIAA2022 in regulating neuronal development and provide insight into the molecular mechanisms underlying intellectual disability and ASD.

Disclosures: J.P. Gilbert: None. H. Man: None.

Poster

474. Dendritic Growth and Branching

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 474.25/A52

Topic: A.04. Axon and Dendrite Development

Support: Japan Grants-in-Aid for Scientific Research 26460250

Takeda Science Foundation

Narishige Neuroscience Research Foundation

Title: Morphological characteristics of cerebellar interneuron Lugaro cells using with GFP-expressing transgenic mice line

Authors: *T. MIYAZAKI1, K. F. TANAKA2, M. WATANABE1;
         1Hokkaid Univ. Sch. Med., Sapporo, Japan; 2Dept. Neuropsychiatry, Sch. Med. Keio Univ., Tokyo, Japan
Abstract: Cerebellar interneuron Lugaro cells (LCs) have fusiform cell bodies and extend horizontal bipolar dendrites beneath the Purkinje cell (PC) layer. Basic morphological properties of LCs are known, however, detailed neuronal characteristics have been unclear due to the lack of useful neurochemical markers. In the present study, we produced a transgenic mouse line, in which green fluorescent protein (GFP) was specifically expressed in LCs by KENGE (knockin-mediated enhanced gene expression by improved tetracycline-controlled gene induction) -tet system. In fluorescent in situ hybridization, most (~80%) LCs expressed both GAD67 and GlyT2 mRNAs as previously reported, however, a subset (~15%) of LCs expressed only GAD67 mRNA. In multiple immunofluorescence and double immunoelectron microscopy using with GFP immunolabeling and neuronal tracing method, LCs received inhibitory inputs from PC axon collaterals and excitatory inputs from climbing fibers (CFs), mossy fibers and ascending axons of cerebellar granule cells. Furthermore, we demonstrated that LCs formed dendritic meshwork beneath PC layer using cerebellar sections cut parallel with the layer. Interestingly, the extent of dendritic meshwork of given LCs was mostly confined to a cerebellar parasagittal zone as visualized by aldolaseC immunolabeling. We also investigated projection pattern of LC axons. LCs projected ascending and transverse axons to the molecular layer, where they formed symmetrical synapses with somatodendritic domain of inhibitory interneurons. Especially, ascending axons intensively innervated the soma of molecular layer interneurons (MLIs). In the developing cerebellum, LCs were generated from the ventricular zone around the birth and migrated to the PC layer with extending their dendrites from the both somatic apical ends. The zone-specific dendritic meshwork was completed by P10. These results suggest that LC receives zone-specific inputs from the early developmental stages and they strongly innervate MLIs on the same parasagittal zone by ascending axons. Considering that the orientation of MLIs and PCs is also organized into the cerebellar zone, LCs may constitute intrazonal microcircuit accompanying with MLIs and PCs, and regulate synchronous disinhibition of PCs on the parasagittal zone.


Poster

474. Dendritic Growth and Branching

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 474.26/A53

Topic: A.04. Axon and Dendrite Development

Title: Neuronal PARP regulates dendritic morphology in developing neurons
**Authors:** *J. Y. HUANG*¹, K. WANG², J. P. ADELMAN², M. S. COHEN¹;
¹Physiol. & Pharmacol., ²Vollum Inst., Oregon Hlth. & Sci. Univ., Portland, OR

**Abstract:** The precise control of dendritic morphology is critical for proper neural circuit formation. Recent studies in mice demonstrate a role for nicotinamide adenine dinucleotide (NAD⁺) in regulating dendritic morphology and cognitive function; however, the downstream effectors of NAD⁺ that mediate these neuronal functions remain unknown. NAD⁺ is not only a cofactor for oxidoreductases, but is also a substrate for a family of enzymes known as poly-ADP-ribose-polymerases (PARPs). These enzymes transfer the ADP-ribose unit from NAD⁺ to amino acids on target proteins, a process known as ADP-ribosylation. ADP-ribosylation is an essential post-translational modification, but it is far less understood compared to other post-translational modifications, such as phosphorylation. To investigate the potential role of PARPs in neuronal development, we first analyzed PARP expression in the brain. One particular PARP, which we refer to as neuronal-PARP (n-PARP), was expressed at high levels in the embryonic rat brain. Knockdown of n-PARP resulted in a significant reduction of dendritic complexity in cultured primary hippocampal neurons and in vivo. Overexpression of n-PARP in primary neurons increased dendritic complexity. Taken together, our results demonstrate a role for n-PARP in the regulation of dendritic morphology during neuronal development. The identification of n-PARP as a critical regulator of dendritic morphology provides the first evidence that ADP-ribosylation plays a role in normal neuronal cell physiology.

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**Poster**

**474. Dendritic Growth and Branching**

**Location:** Hall A

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**Program#/Poster#:** 474.27/A54

**Topic:** A.04. Axon and Dendrite Development

**Support:** AS-103-TP-B05

**Title:** Cortactin binding protein 2 increases microtubule stability and regulates dendritic morphogenesis

**Authors:** *P.-Y. SHIH, Y.-K. CHEN, S.-P. LEE, Y.-P. HSUEH;
Academia Sinica, Inst. of Mol. Biol., Taipei, Taiwan
**Abstract:** Dendritic spines are important subcellular structure for neuron to receive the input from presynaptic neuron. Specifically, dendritic spines are the major locations of excitatory synapses in mammalian brain. Previous studies indicated that the development defects of dendritic spines may result in various neuropsychiatric diseases. Recently, we identified a neuron-specific gene, CTTNBP2, which regulates the formation and maintenance of dendritic spines by regulate Cortactin protein mobility. Besides, CTTNBP2 controls synaptic distribution of the protein phosphatase 2A. Here, we show that in addition to associating with F-actin cytoskeletons via the interaction with Cortactin, CTTNBP2 also associated with microtubules, increased microtubule stability and consequently regulated dendritic development. We identified that the middle (Mid) region of CTTNBP2 associated with microtubules. However, the association with the Mid region is not sufficient for microtubule regulation per se. The homooligomerization through the N-terminal NCC region of CTTNBP2 is also necessary. In cultured hippocampal neurons, knockdown of CTTNBP2 or expression of the Mid or NCC domain alone reduced the acetylation levels of microtubules and impaired early stage dendrite outgrowth and dendritic arborization. Our study suggests that CTTNBP2 influences both the F-actin and microtubule cytoskeletons and thus performs two distinct functions in neuronal morphogenesis: dendritic spine formation and dendritic morphogenesis.

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**Poster**

**474. Dendritic Growth and Branching**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 474.28/A55

**Topic:** A.04. Axon and Dendrite Development

**Title:** Pharmacological blockade of Na+/Ca\(^{2+}\) exchanger modulates the growth and development of Purkinje cell dendritic arbor in mouse cerebellar slice cultures

**Authors:** P. SHERKHANE\(^1\), *J. P. KAPFHAMMER\(^2\);
\(^1\)Dept. of Biomedicine, Univ. of Basel, Inst. of Anat., 4056 Basel, Switzerland; \(^2\)Univ. Basel, 4056 Basel, Switzerland

**Abstract:** Purkinje cells are the principal neurons of the cerebellar cortex and have an extensive and elaborate dendritic tree. Chronic activation of type I metabotropic glutamate receptors severely inhibits Purkinje cell dendritic growth. This effect is mediated by calcium influx through P/Q-type and T-type Ca\(^{2+}\) channels. Furthermore, the blockade of Plasma membrane calcium ATPase 2 (PMCA2) moderately inhibits the growth of the Purkinje cell dendritic arbor.
In contrast, the blockade of PMCA2 rescues the dendritic tree from the drastic reduction normally seen with chronic mGluR1 activation. These findings indicate that influx and extrusion of calcium plays a pivotal role in the development of the Purkinje cell dendritic arbor. The sodium-calcium-exchanger (NCX) is another calcium exchange mechanism in Purkinje cells which can mediate Ca\textsuperscript{2+} and Na\textsuperscript{+} fluxes across the synaptic plasma membrane in a bidirectional mode: the forward mode (Ca\textsuperscript{2+} efflux mode) and reverse mode (Ca\textsuperscript{2+} influx mode). We inhibited these modes by pharmacological treatments to study their effects on the development of Purkinje cell dendritic arbors. The blockade of either the forward mode by Bepridil or the reverse mode by KB-R7943 inhibited the growth and development of the Purkinje cell dendritic tree. However, reverse mode inhibition by KB-R7943 not only strongly reduced the size of the dendritic arbor, but also induced a thickening of the distal dendrites. The combined inhibition of both the modes had a profound negative impact on the dendritic development in Purkinje cells. We have also tested other benzyloxyphenyl derivatives like SEA0400, YM-244769 and SN-6 which preferentially block the reverse mode similar to KB-R7943 in cerebellar slice cultures. They showed similar effects on Purkinje cell dendritic development with their exception of the thickening distal dendrites seen in KB-R7943 treated slice cultures suggesting that this phenotype might be unrelated to blockade of the reverse mode. Our findings show that interfering with the function of the Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger profoundly affects Purkinje cell dendritic growth probably by altering the calcium equilibrium within Purkinje cell dendrites.

Disclosures: P. Sherkhane: None. J.P. Kapfhammer: None.

Poster

474. Dendritic Growth and Branching

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

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Topic: A.04. Axon and Dendrite Development

Support: Smoking Research Foundation

Title: Nicotine facilitates neurite outgrowth of primary cultured cells in superior cervical ganglia (SCG) and PC12 cells

Authors: *S. TAKATORI\textsuperscript{1}, F. TAKAYAMA\textsuperscript{2}, H. HINO\textsuperscript{2}, K. KIMURA\textsuperscript{1}, N. ONO\textsuperscript{3}, H. KAWASAKI\textsuperscript{1};
\textsuperscript{1}Matsuyama Univ., Matsuyama, Japan; \textsuperscript{2}Okayama Univ., Okayama, Japan; \textsuperscript{3}Fukuoka Univ., Fukuoka, Japan
**Abstract:** Background: Our previous report demonstrated that in vivo study nicotine facilitates reinnervation of perivascular sympathetic adrenergic nerves, but not perivascular CGRPergic nerves, which were injured by topical Phenol-application in the rat-mesenteric artery and significantly increased levels of nerve growth factor (NGF) contents in superior cervical ganglia (SCG) and mesenteric arteries, but not dorsal root ganglia (DRG). In this report, we showed that in vivo nicotine produced increased levels of NGF receptor TrkA expression in SCG. Nicotine-induced increases in NGF contents and TrkA expression were inhibited by the nicotinic acetylcholine receptor (nAChR) antagonist hexamethonium pretreatment (Takatori S et al., Eur J Pharmacol, 748: 1-9, 2015.). To clarify possible mechanisms, the present study was investigated whether nicotine affects neurite outgrowth of primary cultured cells in SCG and PC12 cells in vitro. Methods: SCG cells were isolated from Wistar rats and PC12 cells purchased were primarily cultured for 5-7 days. Numbers of neurite outgrowth from cell body were measured in the absent (Control) or presence of nicotine (10-1000 µM), acetylcholine (ACh) (10-1000 µM) or NGF (10-100 ng/mL). Hexamethonium (100 µM) was incubated with nicotine for 5-7 days. Results: In SCG cells, nicotine concentration-dependently increased neurite outgrowth numbers from tyrosine hydroxylase-immunopositive SCG cells. The nicotine-induced increase in neurite numbers was dependent on the exposure time (8 to 24 hr/day) and significantly inhibited by combined incubation of hexamethonium. However, ACh had no effect on the neurite numbers. In PC12 cells, nicotine caused an increase in neurite numbers, which was blocked by hexamethonium (100 µM). NGF also markedly increased neurite growth numbers of SCG and PC12 cells. Conclusion: These results suggest that nicotine has a neurotrophic effect on perivascular adrenergic nerve innervation through activation of nAChR, similar to in vivo assay we previously evaluated.

**Disclosures:** S. Takatori: 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.; Smoking Research Foundation. F. Takayama: None. H. Hino: None. K. Kimura: None. N. Ono: None. H. Kawasaki: 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.; Smoking Research Foundation.

**Poster**

474. Dendritic Growth and Branching

**Location:** Hall A

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**Topic:** A.05. Synaptogenesis and Activity-Dependent Development

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**Title:** Postnatal development of dendritic structure of layer III pyramidal neurons in cerebral cortex of marmoset

**Authors:** *T. SASAKI*¹², T. OGA¹³, H. AOI¹³, I. FUJITA³⁴, N. ICHINOHE¹²;
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**Abstract:** In the primate cerebral cortex, dendritic spines rapidly increase in number after birth up to infancy or mid-childhood, and then decrease towards adulthood. Abnormalities in these processes accompany several psychiatric disorders. We have been investigating the normal processes of spine formation/pruning in the cerebral cortex using the common marmoset as a primate model. In this study, we examined developmental changes of basal dendrites and spines of layer III pyramidal cells in the vision-related areas (prefrontal area 12, temporal area TE, primary visual area V1), and the medial prefrontal cortex (mPFC: granular area 8B/9, dysgranular area 14r, agranular area 24). Cells were intracellularly injected with Lucifer Yellow in lightly fixed slices, and reacted for Diaminobenzidine. Basal dendrites of more than 500 cells were reconstructed, and their morphological features were analyzed. The dendritic field areas and total dendritic length in areas 12, TE and the mPFC areas showed generally similar time course. They increase during the first 2-3 postnatal months, and then slightly decrease (areas 8B/9, 14r) or remain at similar levels (areas 24, 12, TE) until adulthood. In contrast, the change in branching complexity exhibited notable differences between the vision-related areas and the
mPFC areas. While the branching complexity in the vision-related areas monotonically increases up to adulthood, that in the mPFC areas reaches a peak at 2M, and thereafter slightly reduces or remains unchanged. In the six areas, the spine density concurrently increases up to 3M, and subsequently decreases with area-specific patterns. The peak spine density was higher in the mPFC areas than in the vision-related areas at all developmental stages examined. The total spine number of individual cells is higher in the mPFC areas than in the vision-related areas throughout all ages. In these areas, the total spine number was the highest at 3M except for area 14r (the peak occurred at 2M). Synaptogenesis occurs almost synchronously across the cortical areas. The net decrease ratio in the total number of spines is larger in all the granular areas examined (area 8B/9 (43.2%), area 12 (40.7%), TE (49.2%), and V1 (58.7%)) than in agranular area 24 (33.3%) and dysgranular area 14r (28.5%). Thus, cells in less granular areas of mPFC show more modest spine elimination than those in granular areas. These features may be related to the vulnerability of this region to psychiatric disorders. Search for the molecular mechanisms that underlie the developmental changes will provide further clues for an understanding of pathogenesis of developmental disorders.


Poster

475. Neuronal Differentiation: Activity-Dependent Mechanisms

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 475.01/A58

Topic: A.01. Neurogenesis and Gliogenesis

Support: NSFC No. 31271176 and 81171147

Title: Activity-dependent regulation of radial glial cell proliferation by HDAC1 in the developing Xenopus tectum

Authors: *W. SHEN1, Y. TAO2, H. RUAN1, X. GUO1, L. LI2;
1Hangzhou Normal Univ., Zhejiang, China; 2First Affiliated Hosp. of Nanjing Med. Univ., Nanjing, China

Abstract: In the developing central nervous system (CNS), progenitor cells differentiate into progeny to form functional neural circuits. Radial glial cells (RGs) are a transient progenitor cell type that is present during neurogenesis. It is thought that a combination of neural trophic factors, neurotransmitters and electrical activity regulates the proliferation and differentiation of
RGs. However, it is less clear how epigenetic modulation controls the proliferation of RGs in the developing brain. We sought to explore the effect of visual activity and histone deacetylase (HDAC) on the proliferation of RGs in the visual optic tectum of Xenopus laevis. We used the thymidine analog BrdU (5-bromo-2'-deoxyuridine) with anti-BrdU antibody to measure the cell proliferation and brain lipid-binding protein (BLBP) antibody to label RGs in the whole-mount optic tectum. We found that the number of BrdU-labeled precursor cells along the ventricular layer of the tectum decrease developmentally from stage 46 to stage 49. The co-labeling of BrdU- and BLBP-positive cells showed that the majority of BrdU-labeled cells along the tectal midline are RGs. BLBP-positive cells are also developmentally decreased with the maturation of the brain. Furthermore, HDAC1 expression is developmentally down-regulated in tectal cells, especially in the ventricular layer of the tectum. Pharmacological blockade of HDACs using Trichostatin A (TSA) or Valproic acid (VPA) decreased the number of BrdU-positive, BLBP-positive and co-labeling cells. Specific knockdown of HDAC1 by a morpholino (HDAC1-MO) decreased the number of BrdU- and BLBP-labeled cells and increased the acetylation level of histone H4 at lysine 12 (H4K12). We also found that visual deprivation (VD) induces the increase of BrdU-positive precursor cells and BLBP-positive RGs. The VD-induced increase in precursor cells was blocked by HDAC1 knockdown with HDAC1-MO at stage 49 tadpoles. These data demonstrate that the proliferation of RGs are visual activity-dependent and HDAC1 regulates radial glia cell proliferation in the developing optical tectum of Xenopus laevis.

Disclosures: W. Shen: None. Y. Tao: None. H. Ruan: None. X. Guo: None. L. Li: None.

Poster

475. Neuronal Differentiation: Activity-Dependent Mechanisms

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 475.02/A59

Topic: A.01. Neurogenesis and Gliogenesis

Support: BELSPO IAP

WELBIO

BOF UHasselt

Title: Disruption of cortical circuitry development in glycine receptor alpha 2 knockout mice

Authors: *J.-M. RIGO, G. MORELLI, A. AVILA, N. AOURZ, I. SMOLDERS, B. BRÔNE, L. NGUYEN;
Abstract: Previous studies have revealed an important role of strychnine-sensitive glycine receptors (GlyRs) in the cerebral cortex during neurogenesis. Specifically, absence of GlyR alpha 2 subunits in a genetically disrupted mouse model leads to defects in the interneuronal migration and proliferation of projection neurons. In order to evaluate the long-term consequences of these early defects, we examined the role of GlyRs during postnatal development of the cerebral cortex. Remarkably, GlyR alpha 2 knockout mice displayed a significant reduction in the number of parvalbumin positive interneurons at postnatal day 14 associated with a reduction of upper layer and layer V projection neurons of the cortex. Moreover, morphological and synaptic defects were assessed in the cerebral cortex of knockout mice by performing 3D reconstruction of single neurons and whole-cell patch-clamp recordings. Morphological studies of biocytin-filled neurons revealed altered dendrites growing and generation of spines. Furthermore, whole-cell recordings showed a substantial decrease in the frequency of inhibitory post-synaptic currents (IPSCs) and increase of excitatory postsynaptic currents (EPSCs). These preliminary findings are consistent with the idea that GlyRs might have an important role during the development of the cortical circuitry.

Abstract: During rat midbrain (MB) formation, dopamine neuron differentiation occurs between embryonic days (E) 9 to 15. During this process, the modifications of epigenetic marks in histones and DNA are essential for the expression of genes associated to the dopaminergic phenotype. The DNA demethylation process has an important role during neurogenesis, since increases of 5-hydroxymethylcytosine (5hmC) have been associated to transcriptional activation of neuronal genes. Recently, it was demonstrated that an injection of histamine (HA) in E12 decreases the number of mesencephalic dopamine neurons. In this work, we analyzed the presence of histamine receptors in vivo at two different stages: neural precursor cells and differentiated neurons. Next, we performed intrauterine injections at representative stages of MB neurogenesis (E10, E12, E14 and E16) to investigate if HA precludes the expression of Tyrosine Hydroxylase (TH), the rate limiting enzyme for dopamine synthesis. We found that embryos injected at E10 and E12 showed a marked decrease in TH staining, strongly suggesting that HA is acting on neural progenitors. To analyze a possible HA action mechanism, we looked for changes in the patterns of modified cytosines in the Th gene by bisulfite conversion and sequencing. Although we found a similar global percentage of modified cytosines in HA-treated embryos compared to controls, we found specific changes in the first intron of this gene. Furthermore, we analyzed the presence of 5hmC and 5-methylcytosine (5mC) marks, and found that HA injection decreased significantly the percentage of 5mC and 5hmC at intron 1 of Th gene to 52% and 63%, respectively, compared to vehicle-injected embryos. Surprisingly, when the pluripotency gene Oct4 was analyzed, we found that HA decreased significantly the percentage of 5mC to 46%, compared to controls. In the case of the housekeeping gene Gapdh, we did not find cytosine modifications in control embryos, nor in those treated with HA. Finally, we found that HA have a long-term effect on the dopaminergic system, since the administration of HA at E12 decrease neuronal fibers positive to TH six days after the injection. These findings will help us understand the molecular effects of HA during differentiation of dopamine neuron and to design strategies to counteract dopaminergic degeneration, responsible for Parkinson’s disease. Supported by PAPIIT IN208713 and CONACyT CB09/131281.

Title: Histamine impairs midbrain dopaminergic development \textit{in vivo} by activating histamine type 1 receptors

Authors: *I. ESCOBEDO;*  
Univ. Nacional Autónoma De México, México, Mexico

Abstract: Histamine (HA) regulates the sleep-wake cycle, synaptic plasticity and memory in adult mammals. Dopaminergic specification in the embryonic ventral midbrain (VM) coincides with increased HA brain levels. To study the effect of HA receptor stimulation on dopamine neuron generation, we administered HA to dopamine progenitors, both \textit{in vitro} and \textit{in vivo}. Cultured embryonic day 12 (E12) VM neural stem/progenitor cells expressed transcripts for HA receptors H1R, H2R and H3R. These undifferentiated progenitors increased intracellular calcium upon HA addition. In HA-treated cultures, dopamine neurons significantly decreased after activation of H1R. We performed intrauterine injections in the developing VM to investigate HA effects \textit{in vivo}. HA administration to E12 rat embryos notably reduced VM Tyrosine Hydroxylase (TH) staining 2 days later, without affecting GABA neurons in the midbrain, or serotonin neurons in the mid-hindbrain boundary. qRT-PCR and Western blot analyses confirmed that several markers important for the generation and maintenance of dopaminergic lineage such as TH, Lmx1a and Lmx1b were significantly diminished. To identify the cell type susceptible to HA action, we injected embryos of different developmental stages, and found that neural progenitors (E10 and E12) were responsive, whereas differentiated dopaminergic neurons (E14 and E16) were not susceptible to HA actions. Proliferation was significantly diminished, whereas neuronal death was not increased in the VM after HA administration. We injected H1R or H2R antagonists to identify the receptor responsible for the detrimental effect of HA on dopaminergic lineage and found that activation of H1R was required. These results reveal a novel action of HA affecting dopaminergic lineage during VM development.

Disclosures: I. Escobedo: None.

Poster

475. Neuronal Differentiation: Activity-Dependent Mechanisms

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 475.05/A62

Topic: A.01. Neurogenesis and Gliogenesis
Support: NIGMS RO1 GM072005-01

BK21 Plus Creative Innovation Group for Leading Future Functional Food Industry

Title: Effects of calcium influx on interleukin-6-mediated neuronal differentiation of neural progenitor cells

Authors: *J. OH; Kyungpook Natl. Univ., Daegu, Korea, Republic of

Abstract: Effects of calcium influx on interleukin-6-mediated neuronal differentiation of neural progenitor cells

Jisun Oh¹, Jong-Sang Kim¹, Michael A. McCloskey² and Donald S. Sakaguchi² ¹School of Food Science and Biotechnology (BK21 plus), Kyungpook National University, Daegu 702-701 Republic of Korea ²Department of Genetics, Development and Cell Biology, Iowa State University, Ames, IA 50011

Abstract

The purpose of this study was to investigate possible molecular mechanisms through which interleukin-6 (IL-6) induces neuronal differentiation of adult hippocampal neural progenitor cells (AHPCs). We previously reported that astroglial cells from neonatal rat brain produce soluble factors which enhance neuronal differentiation of AHPCs, and that IL-6 is an astrocyte-derived factor which specifically promotes neuronal differentiation of AHPCs. In the present study, we examined the influence of extracellular calcium and voltage-gated calcium channel antagonists on IL-6-induced neuronal differentiation of AHPCs. AHPCs were cultured in various concentrations of external calcium or with L-type voltage-gated calcium channel blockers. Our results demonstrate that extracellular calcium was critical for neuronal differentiation of IL-6-treated AHPCs, that a blockage of L-type Cav channels inhibited IL-6-enhanced neuronal differentiation of AHPCs, and that exogenous IL-6 treatment increased the percentage of AHPCs immunoreactive for an activated form of CREB (phosphorylated CREB). These results suggest that IL-6 may induce AHPCs to commit to a neuronal lineage by promoting extracellular calcium influx which leads to CREB activation. Our findings may provide insight into understanding injury-induced neurogenesis and have implications for developing cell-based therapeutic strategies using adult neural progenitor cells.

Disclosures: J. Oh: None.

Poster

475. Neuronal Differentiation: Activity-Dependent Mechanisms

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 475.06/A63
Topic: A.01. Neurogenesis and Gliogenesis

Support: A07376

Title: Non-synaptic communication during early cortical column formation revealed by genetically encoded Ca$^{2+}$ indicators

Authors: *B. G. RASH$^1$, J. B. ACKMAN$^2$, P. RAKIC$^{1,2}$; $^1$Dept. of Neurobiol., Yale Univ., New Haven, CT; $^2$Kavli Inst. for Neurosci. at Yale, New Haven, CT

Abstract: Cortical columns are basic cellular and functional units of the cerebral cortex and are thought to be malformed in many brain disorders, but how they initially form is not well understood. Using the genetically encoded calcium (Ca$^{2+}$) indicator, GCaMP5G, in the mouse embryonic forebrain we demonstrate that Ca$^{2+}$ fluxes propagate bi-directionally within the elongated fibers of radial glial cells (RGC)s, providing a novel communication mechanism linking the stem cell and differentiative niches prior to synaptogenesis. Correlated activity in RGC fiber clusters heralded the development of early cortical columns, and high frequency Ca$^{2+}$ transients featured prominently during initial neurogenesis and coordinated neuronal migration to the appropriate cortical columns. Furthermore, radially-oriented Ca$^{2+}$ signaling was induced by Notch and fibroblast growth factor activity implicated in cortical expansion. Therefore, interaction of genetic and multiple Ca$^{2+}$-dependent mechanisms shapes the formation of cortical columns by influencing the timing of neuronal genesis and mode and rate of migration. Finally, we show that prenatal exposure to Ca$^{2+}$ activity-blocking drugs led to defects in cortical columns and connectivity.

Disclosures: B.G. Rash: None. J.B. Ackman: None. P. Rakic: None.

Poster

475. Neuronal Differentiation: Activity-Dependent Mechanisms
Title: Underlying mechanisms of learning: the contribution of mature spines and GluA2 expression in CA3

Authors: *A. ALLIGER*1, A. AUBRY2, P. A. SERRANO1;
1Psychology, Hunter Col., New York, NY; 2Psychology, CUNY Grad. Ctr., New York, NY

Abstract: Animals housed in environmental enrichment (EE), even for short or intermittent exposure periods, have shown robust morphological and biochemical changes compared to standard housed (SH) animals. Morphological factors of subjects housed in EE include but are not limited to increases in synaptic proteins and spinogenesis throughout the hippocampus. While total number of spines may be an important determinant of learning and memory, we are now recognizing that type of spine may also broaden our understanding of the neurological mechanisms of these behaviors. By combining Golgi staining with immunohistochemistry, we are able to quantify spine morphology and expression of synaptic proteins (GluA2 and PSD-95) within spines utilizing IMARIS software. After six weeks of EE, we measured 1) spatial learning via the radial arm maze (RAM), 2) spine density for filopodia, stubby, long-thin and mushroom spines and 3) the clustering of synaptic markers GluA2, and PSD95 in area CA3 of juvenile rats. Four treatment conditions of EE/no RAM, SH/no RAM, EE/RAM, and SH/RAM allowed comparisons between the expression of spine types and synaptic markers. For spatial learning assessment, animals were given three days of RAM training (10 trials per day) where 4 of 8 arms were baited. EE subjects performed significantly better on RAM over SH subjects measured with percent correct scores. A two-way (RAM x Housing) ANOVA revealed that EE/RAM animals exhibited a significant increase in mushroom spines compared to EE/no RAM, SH/no RAM and to SH/RAM animals. Separate two-way ANOVAs were run for the expression of GluA2 and PSD-95 in mushroom spines. The co-localization of GluA2 and PSD-95 increased in EE/RAM animals compared to SH/no RAM and SH/RAM animals. Furthermore, EE/RAM animals showed an increase in the expression of GluA2, PSD-95, and their colocalization in mushroom spines compared to EE/no RAM animals. A lack of increase of long-thin spines may be due to a possible ceiling effect of the already high levels of long thin spines established during the housing of EE. These results suggest that while the density of mature spine types increase
(including higher levels of synaptic GluA2 and PSD-95 expression) there is a decrease in immature spines during spatial learning. This is supported with previous results from our lab showing that EE prior to RAM increases filopodia and its expression of synaptic GluA2. Given that filopodia are thought to be pre-cursors to mature spines, our results suggest that as EE animals acquire spatial memory, filopodia mature into long-thin and mushroom spines. The increase in mature spine types may represent an underlying mechanism by which EE improves learning.

Disclosures:  A. Alliger:  None.  A. Aubry:  None.  P.A. Serrano:  None.

Poster

475. Neuronal Differentiation: Activity-Dependent Mechanisms

Location:  Hall A

Time:  Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#:  475.08/A65

Topic:  A.01. Neurogenesis and Gliogenesis

Support:  Basil O'Connor Award

March of Dimes Foundation

Klingenstein Foundation Award in Neuroscience

NSF

NIH-NINDS

Shriners Hospital for Children

Title:  Sonic hedgehog regulates motor neuron phenotype through calcium-dependent electrical activity in the embryonic spinal cord

Authors:  *Y. H. BELGACEM, K. A. SPENCER, L. BORODINSKY;  
UC Davis, Sacramento, CA

Abstract:  Spinal cord injury is a debilitating condition affecting up to half million people every year in the world. Among the consequences of spinal cord trauma is the loss of motor neuron innervation of the skeletal muscle, highlighting the importance of devising therapeutic strategies for motor neuron regeneration. During nervous system development, spontaneous electrical activity generates a temporal window of plasticity important for neuronal differentiation and maturation. We have previously discovered that Sonic hedgehog (Shh), in the embryonic spinal
cord, controls spontaneous Ca\(^{2+}\)-mediated electrical activity in neurons through a novel non-canonical signaling pathway. In this study, we investigate the role of the Shh-Ca\(^{2+}\) signaling pathway on spinal motor neuron differentiation in *Xenopus leavis* embryos. We hypothesize that Shh, via Ca\(^{2+}\)-dependent electrical activity increases the number of motor neurons. We implanted beads loaded with drugs to manipulate Ca\(^{2+}\)-dependent Shh pathway in *Xenopus* embryos right after closure of the neural tube and processed tadpoles, 24 h later, for immunostaining. Results show that enhancing Shh signaling or Ca\(^{2+}\) spikes with SAG or veratridine, respectively, increases the number of cells expressing the motor neuron transcription factor HB9. In contrast, when inhibiting Shh signaling or suppressing Ca\(^{2+}\) spikes the motor neuron number decreases. Occlusion experiments reveal that Ca\(^{2+}\) spikes are downstream Smo activation in the regulation of motor neuron phenotype. To determine the molecular mechanisms underlying motor neuron specification driven by the Shh-Ca\(^{2+}\)-signaling, we search for potential activity-responsive elements within the 5' conserved regulatory region of the human hb9 gene shown to control hb9 expression. We found a cAMP-responsive element (CRE)-like sequence and an activator protein 1 (AP1) recognition site. We sub-cloned a 270 bp fragment containing these activity-responsive elements upstream of the firefly luciferase gene. Injecting this reporter construct in embryos shows, as expected, expression of luciferase in the motor neuron domain of the spinal cord. Results show that enhancing Ca\(^{2+}\) spike activity by either overexpressing Na\(_{\text{v}}\)2a or by incubating dissected spinal cords with veratridine, enhances luciferase expression. Mutating the CRE-like sequence had no effect on the activity-induced increase in luciferase expression, while mutating the putative AP1 site abolished this effect. Altogether, these results suggest that Shh regulates motor neuron phenotype by acting directly in neurons through a novel mechanism implicating electrical activity.

**Disclosures:** Y.H. Belgacem: None. K.A. Spencer: None. L. Borodinsky: None.

**Poster**

475. Neuronal Differentiation: Activity-Dependent Mechanisms

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 475.09/A66

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

**Support:** NIH Grant 1 R15 NS067566 to Margaret S Saha

2015 Dintersmith Honors Fellowship to Eileen Ablondi

**Title:** The roles of gad1.1 regulation and calcium transients in neurotransmitter phenotype specification
Abstract: The acquisition of the appropriate balance of excitatory and inhibitory neurons is a vital step in the development of a functional nervous system, with misregulation of this balance implicated in disorders ranging from epilepsy to autism. While this system has been well-characterized in adult organisms, the mechanisms by which this phenotype is acquired during development are less clearly understood. In order to gain a more complete understanding of this process, we have analyzed the roles played by both 'hard-wired' transcriptional cascades and activity-dependent developmental processes. The former involves investigating the transcriptional activation of gad1.1, a gene which codes for the rate-limiting enzyme for GABA synthesis. GABA (gamma aminobutyric acid) is the primary inhibitory neurotransmitter in the vertebrate nervous system, and is therefore of great relevance to the eventual acquisition of appropriate excitatory/inhibitory patterning. Using Xenopus laevis as a model organism, we have used promoter analysis techniques to characterize the functional role of the upstream promoter region of gad1.1, and have identified distinct expression patterns under the control of various construct lengths. Additionally, sequence analysis methods have been utilized to identify evolutionarily conserved regions in this sequence through comparisons between Xenopus laevis individuals as well as individuals representing closely-related species, with the intention of identifying transcription factors involved in the process of neurotransmitter phenotype specification. We have also investigated a second major mechanism of neuronal phenotype acquisition, the spontaneous calcium transients that occur within developing neural cells. Previous research in the field has linked characteristic patterns of developmental intracellular calcium spikes to specific neuronal fates. Our lab has found no correlation between spike frequency and neurotransmitter phenotype; however, we are exploring novel methods of data analysis to investigate the relationship between neurotransmitter phenotype and general calcium activity patterns.

Title: Environmental temperature influences spinal neuron differentiation in vivo

Authors: *K. A. SPENCER, L. N. BORODINSKY; Univ. of California Davis, Sacramento, CA

Abstract: During development differentiation of neurons is necessary for establishing the cell populations that will make-up the neuronal network in the mature nervous system. Embryonic Ca2+ activity modulates neuronal differentiation and we are interested in how the environment may influence this activity and the specification of neuronal populations. It is well established that environmental temperature regulates the rate of development in ectotherms, yet the specific impact temperature has on nervous system development is unknown. The purpose of the following study is to investigate how temperature influences spinal neuron differentiation in embryonic Xenopus laevis. We chose to examine the sensory and motor neuron populations in animals grown at different temperatures (14.5, 22.5 and 26.5°C) after reaching identical tadpole stages (stage 40). Immunostaining for HNK1, a membrane glycoprotein expressed in Rohon-Beard sensory neurons, indicates an increase in sensory neuron number in embryos grown at cold and warm temperatures compared to those grown at room temperature. Staining for Hb9, a transcription factor necessary for motor neuron maintenance, shows a 2-fold increase in the number of motor neurons in embryos raised at cold temperature compared to those raised at warm and room temperatures. Further analysis of morphological features of both sensory and motor neurons in embryos grown at different temperatures is underway. To investigate the mechanisms underlying these temperature-driven changes in neuronal specialization, we assessed the levels of spontaneous Ca2+ spike activity in embryonic spinal neurons. Previous results show that in the embryonic ventral spinal cord, Ca2+ spike frequency increases 2-fold in response to acute exposure to a cold temperature (14.5°C) compared to room temperature. In order to determine whether the acute change in activity persists, embryos expressing GCaMP6 were grown at different temperatures and time-lapse imaged at those temperatures. Preliminary results suggest that chronic exposure to a cold temperature also elicits an increase in Ca2+ spike frequency in the ventral spinal cord. Altogether these results show that temperature driven changes in Ca2+ spike activity are rapid, suggesting a non-transcriptional mechanism, and
appear to endure. Temperature regulation of sensory and motor neuron differentiation may depend on the changes in embryonic Ca2+ activity. The findings of this investigation suggest that the environment intervenes in the differentiation program of developing neurons to allow for the establishment of the best-equipped neuronal circuit for responding to the changing surroundings.

Disclosures: K.A. Spencer: None. L.N. Borodinsky: None.

Poster

476. Opiate, Cytokines, and Other Neuropeptides

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 476.01/A68

Topic: B.01. Neurotransmitters and Signaling Molecules

Title: Association of nitric oxide with the induction of interleukin 1 beta in microglia

Authors: *K. NAKAJIMA1, K. SUDO1, Y. TAKEZAWA1, S. KOHSAKA2; 1Soka Univ., Tokyo, Japan; 2Natl. Inst. of Neurosci., Tokyo, Japan

Abstract: Microglia in vitro induced an inflammatory cytokine interleukin 1beta (IL-1β) together with nitric oxide (NO) and superoxide anion (O2-) by stimulation with lipopolysaccharide (LPS). In this study, we investigated the role of NO or O2- in the signaling mechanism by which IL-1β is induced in microglia. The LPS-inducible IL-1β was significantly suppressed by pretreatment with NO scavenger 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl 3-oxide, but not by O2- scavenger N-acetyl cystein, suggesting the close association of NO with IL-1β induction. In fact, the pretreatment of microglia with inducible NO synthase inhibitor 1400w prior to LPS stimulation significantly reduced the production of IL-1β, and the addition of NO-donor S-nitroso-N-acetyl-DL-penicillamine (SNAP) into microglia led to the induction of IL-1β. These results suggested that NO induces IL-1β through a specific signaling cascade. LPS-dependent IL-1β induction was significantly suppressed by extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK) inhibitors and nuclear factor kappaB (NFkB) inhibitor, indicating that these ERK/JNK and NFkB serve in the cascade of IL-1β induction. As expected, ERK/JNK and NFkB were all activated in the SNAP stimulated microglia. Taken together, these results indicated that NO is an important signaling molecule for activating ERK/JNK and NFkB, whose activations are requisite to induce IL-1β in microglia.

Disclosures: K. Nakajima: None. K. Sudo: None. Y. Takezawa: None. S. Kohsaka: None.
Peripheral local activation of oxytocin receptors inhibits the nociceptive activity of the spinal dorsal horn wide dynamic range neurons

**Abstract:** The best described functions of the oxytocin are related to hormonal aspects associated to uterine contraction during parturition, lactation and more recently for its role in social behavior, but several studies have suggested that this neuropeptide could act as a neuromodulator in the endogenous analgesia. Indeed, endogenous release of oxytocin upon the spinal dorsal horn after paraventricular nucleus stimulation inhibits selectively the nociceptive neuronal activity of Aδ- and C-fibers, an effect mediated by oxytocin receptors. In this sense, some reports suggest the expression of oxytocin receptors at peripheral level, but to our knowledge no study has yet reported a particular function of these receptors. The aim of the present study was to investigate in electrophysiological recordings, behavioral nociception tests and neuronal tracing the potential antinociceptive effect of peripheral activation of oxytocin receptors (OTR). In anaesthetized (2-3 % sevofluorane) male Sprague-Dawley rats we test the nociceptive responses evoked on the wide dynamic range (WDR) neurons at the lumbar segments (L3-L6) of the spinal cord. In these animals, extracellular unitary recordings were performed (4-8 MΩ). The nociceptive neuronal responses were evoked by 20 electrical stimuli (0.5 Hz, 1-ms pulse duration, 0.1-3.3 mA) delivered on the receptive field located in the ipsilateral (to recording site) hindpaw. Furthermore, in order to test the behavioral effects of peripheral actions of oxytocin on nociception we did experiments using the 1% formalin nociception test (50 µl formalin injected s.c. into the dorsal surface of the hindpaw). In both cases (electrophysiological and behavioral experiments) a dose-response curves were made. Additionally, using the neuronal tracers Fluoro-Gold® and Fluoro-Rubi® injected in the sciatic nerve we labelled the peripheral neuronal terminals; this tissue was also prepared to immunofluorescence to OTR, SP, CGRP and IB4. Our results show that peripheral local
administration (s.c.) of oxytocin is able to inhibit the neuronal activity of Aδ- and C-fibers but not Aβ-fibers. Furthermore, the formalin-induced nociception was blocked by oxytocin. In both cases, the effect was antagonized by the L-368,899 (OTR antagonist). Finally, we found that OTR is localized in the terminal neuronal endings localized in the hindpaw. Together, our results suggest that the peripheral OTR activation have a potential antinociceptive effect.


Poster

476. Opiate, Cytokines, and Other Neuropeptides

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 476.03/A70

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: Nanyang Technological University

Title: Diverse postsynaptic signals produced by presynaptic somatostatin interneurons in the claustrum

Authors: *Y. TANG*1, G. J. AUGUSTINE2;

1Lee Kong Chian Sch. of Med., 2Nanyang Technological Univ., Singapore, Singapore

Abstract: Although it is well-established that somatostatin (SST) interneurons produce rapid inhibitory actions on their targets via postsynaptic GABAA receptors, it is not known what role co-release of SST may play in synaptic signalling. We have examined this by characterizing the postsynaptic actions of SST neurons in the claustrum. SST neurons were photostimulated in brain slices prepared from transgenic mice expressing Volvox channelrhodopsin-1 or Chlamydomonas channelrhodospin-2 (Front. Neural Circ. 7:160). Repetitive photostimulation (100 ms, 5 Hz, 5 s train) produced sustained activation of claustral SST neurons, while whole-cell patch clamp recordings measured resulting responses in other claustral neurons. In addition to inhibitory postsynaptic currents mediated by GABAA receptors, two changes in postsynaptic excitability were observed. Action potential duration was broadened in 48% of neurons, while the ability to fire trains of action potentials in response to depolarizing current pulses (1 s duration) was reduced in another 23% of neurons, changing their firing pattern from tonic to phasic. Both of these effects on excitability were long-lasting, persisting for 60 min or longer. The effect on repetitive firing is mediated by GABAB receptors, because this effect was eliminated by a GABAB receptor antagonist (GCP 52432) and was mimicked by a GABAB
receptor agonist (baclofen). SST is responsible for the prolongation of action potential duration, because this effect was blocked by a SST receptor antagonist (cyclosomatostatin) and was mimicked by application of SST. In summary, SST interneurons generate multiple postsynaptic signals, with GABA receptors responsible for acute effects on postsynaptic membrane potential, while GABAB and SST receptors modulate different features of neuronal excitability. This diversity of signalling modalities potentially imparts great flexibility to circuits in which SST interneurons participate.


Poster
476. Opiate, Cytokines, and Other Neuropeptides

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 476.04/A71

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NIH Grant R01 HL098589

Title: Neuropeptide Y modulation of guinea pig intrinsic cardiac neurons

Authors: K. LUCKETT, E. A. POWERS, *J. C. HARDWICK; Ithaca Col., Ithaca, NY

Abstract: Chronic heart disease, such as myocardial infarction (MI), produces remodeling of the autonomic nervous system, and induces an increase in sympathetic output, as well as remodeling of the intrinsic cardiac nervous (ICN) system located within the heart. Sympathetic fibers innervate the parasympathetic intracardiac neurons of the ICN and thus, increased sympathetic activity could lead to altered function within the cardiac plexus. To examine this possibility, we looked at the responses of parasympathetic cardiac neurons to the application of sympathetic neurotransmitters norepinephrine (NE) and neuropeptide Y (NPY). MI was surgically-induced in guinea pigs by ligation of the a branch of descending coronary artery on the left ventricle. After a four week recovery period, whole mounts of the intrinsic cardiac plexus were prepared for intracellular voltage recording, followed by immunohistochemical analysis. NE, NPY, Y1R and Y5R agonists were applied by local pressure ejection to individual neurons impaled with sharp intracellular electrodes for voltage recording. In control animals, NE and NPY each produced a small increase in neuronal excitability and simultaneous application of both substances did not differ from NE alone. NPY application and receptor-specific agonists demonstrated the presence of multiple NPY receptor isoforms present on the intracardiac neurons. In animals with MI, NPY...
responses were blunted while NE responses were enhanced. However, simultaneous application of both NE and NPY showed a significantly greater effect in neurons from MI animals than was seen in control animals. IHC demonstrated NPY-positive fibers and cells within the cardiac plexus and the relative number of NPY-positive cells increased with MI. Taken together, these results demonstrate that MI, which has been shown to increase sympathetic output, alters neuronal expression and responses to NPY within the parasympathetic intrinsic cardiac plexus.


Poster

476. Opiate, Cytokines, and Other Neuropeptides

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 476.05/A72

Topic: B.01. Neurotransmitters and Signaling Molecules

Title: Plasma opiates in subjects with chronic low back pain treated with Transcutaneous Electrical Nerve Stimulation

Authors: *C. I. EZEMA;
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Abstract: Chronic low back pain (CLBP) is a well recognised cause of disability and absences from work. Transcutaneous electrical nerve stimulation (TENS) is being used for pain management in physiotherapy, but its mechanism of action remains controversial. This study investigated the plasma levels of beta-endorphin (BE), met-enkephalin (ME), prostaglandin E2 (PE2) and pain intensity among adult subjects suffering from chronic low back pain (CLBP) who were exposed to TENS. The study involved 63 age-matched adults, distributed into three groups, A, B and C. Subjects in group A were 32, had no CLBP and were exposed to burst modulation TENS, those in group B were 15, had CLBP and were exposed to burst modulation TENS, while those in group C were 16, had CLBP and exposed to placebo TENS. The TENS electrodes were placed at L1 and L5 for 30 minutes. At the beginning of the exposure (0 hr), and 1hr, 24 hrs, and 48 hrs after TENS exposure, blood samples were collected from the subjects for plasma BE, ME, PE2 assay using ELISA, while pain intensity was assessed using Numerical Pain Rating Scale. Analysis of variance (ANOVA) and Pearson’s correlation tests were used in data analysis, level of significance set at p < 0.05. Results showed there was no significant difference in plasma levels of BE, ME and PE2 among the groups (p > 0.05), but pain intensity in group B was significantly lower than in group C only after 1 hr of exposure to TEN (p < 0.05). The findings suggest that the TENS in low back pain does not affect the levels of plasma opiates. This study
was not sponsored by any organisation or individual. **Key words:** Beta-endorphin, met-enkephalin, prostaglandin E2, chronic low back pain, transcutaneous electrical nerve stimulation.

**Disclosures:** C.I. Ezema: None.

**Poster**

**476. Opiate, Cytokines, and Other Neuropeptides**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 476.06/A73

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

**Support:** PAPIIT: TA200314

**Title:** Sedative effect of a neuropeptid obtained from the posterior salivary gland of the mexican red octopus (Octopus maya) in mice CD1

**Authors:** E. GARCÍA -RAMÍREZ1, *A. G. MARTINEZ2, R. BUSTAMANTE-GARCÍA1, S. RODRÍGUEZ-MORALES3;

1Biochem., 2Fac Quimica, UNAM, Mexico City, Mexico; 3Biol., UNAM. Facultad de Química, Mexico city, Mexico; 4SISAL, UNAM. SISAL, Mexico city, Mexico

**Abstract:** In recent years the search for peptide mediators such as neuropeptides (NP) grows exponentially because they are involved in diverse functions as nociception, analgesia, sedation, immune system, just to name a few. In the case of Octopus maya (O. maya), bioactive molecules with neurotoxic activity causing paralyzing effect from its posterior salivary gland (PSG) were extracted. Bioactives molecules from total soluble fraction were fractioned and a purified NP (in process of sequencing) was isolated. The NP was tested in order to identify sedative-like effects in CD1 mice using 7 groups with n = 6 mice (25± 5.0 g), doses 0.25, 0.5, 1.0, 2.0, 4.0, 8.0 and 100 mg/kg were administered i.p. Sedative-like effect was evaluated in 30 min, if the effect was observed a recovery time was recorded. The ED50 was calculated at 0.736 mg/kg by a probit analysis. At small dose (0.25 and 0.5mg / kg) abdominal contraction effect was observed but there was not sedative-like effect. The effect was detected between 15-30 min and the duration of the effect was up to 8 hours. The results shown that O. maya NP has a sedative-like effect around 1 mg/kg and below this dose, abdominal contraction and lower limbs effects were observed, after 24 and 72 h of observation neither adverse effects or death were presented. All animals had with free access to water and maintained under conditions indicated in the NOM 062-ZOO-1999 and with international specifications for the production, care and use of laboratory animals standards.
**Abstract:** Opioid receptors are expressed pre- and postsynaptically throughout the striatum, in addition to MSNs expressing endogenous opioid peptides. The kappa-opioid receptor system has both a large role in stress and stress-induced reinstatement for drug seeking. The role of kappa in drug addiction has been unique compared to the other opioids (mu and delta). In order to better understand the roles of kappa receptors (KORs) in modulating dopaminergic neurotransmission, we investigated the effects on the evoked dopamine release in the mouse striatal slices monitored with fast-scan cyclic voltammetry and amperometry. We found that there was no detectable tonic activation of KORs in the dorsal striatum (dSTR) whereas there was a tonic activation of KORs in the nucleus accumbens (NAcc) shell since selective KOR antagonist nor-binaltorphimine (nor-BNI) significantly enhanced dopamine release evoked by single pulse stimulation. Selective KOR agonist BRL52537 inhibited dopamine levels to 42 ± 5% of control in the dSTR and 49 ± 6% in the shell respectively. To determine the pathways/mechanisms behind inhibition of dopamine levels through KORs, we pretreated the slices with GABA receptor and nACh receptor blockers. In the presence of the blockers, BRL52537 inhibited dopamine release to a similar degree in the shell whereas different in the dSTR. We then used whole-cell patch recording to examine the modulation effect of KORs on cholinergic interneurons. Consistently, neither BRL52537 nor nor-BNI had any effect on the firing of cholinergic interneurons in the shell. In contrast, BRL52537 increased firing of cholinergic interneurons in the dSTR whereas nor-BNI decreased the firing. These data suggest that endogenous dynorphin opioids could inhibit dopamine level through activation of KORs on the dopamine terminals directly in the shell,
whereas the control of the dopamine level by KORs in the dSTR is a combination of action on dopamine terminals directly and through cholinergic interneurons indirectly.

**Disclosures:** **Q. Qin:** None. **T. Westergard:** None. **L. Chen:** None. **G. Li:** None. **H. Zhang:** None.

**Poster**

**476. Opiate, Cytokines, and Other Neuropeptides**

**Location:** Hall A  
**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM  
**Program#/Poster#: 476.08/A75  
**Topic:** B.01. Neurotransmitters and Signaling Molecules  
**Support:** National Natural Science Foundation of China 31271166  
National Natural Science Foundation of China 81471346  
Chongqing Natural Science Foundation cstc2012jjA0405  
**Title:** Orexin/hypocretin exerts postsynaptic excitatory effects and inhibits presynaptic excitatory inputs on reticulospinal neurons of caudal pontine reticular nucleus  
**Authors:** *J. ZHANG, N. YANG, Q.-C. QIAO;  
Third Military Med. Univ., Chongqing, China  
**Abstract:** Orexin, also called hypocretin, is produced by a specific group of neurons located in lateral hypothalamic/perifornical area. This neuropeptide has been substantially implicated in several basic physiological functions, such as the sleep/wake states and reward processes. Interestingly, our previous studies have provided evidence that orexin also plays an important role in motor control through directly exciting neurons in lateral vestibular nucleus (LVN). LVN has been well acknowledged to take an active part in the regulation of body balance and posture in a feedback fashion. In order to fulfill the motor function, however, the feedforward motor control mediated by reticulospinal descending system must coordinate with the LVN-mediated feedback motor control to orchestrate an integrated regulation of body balance and posture. Whether there is a direct modulation of orexin system on reticulospinal descending system-mediated motor control remains unknown. In the present study, therefore, the nervous circuitry of the caudal pontine reticular nucleus (PnC), a part of the reticulospinal descending system, was put into investigation. First, immunofluorescence results showed that orexin receptors are presented in the PnC in rats. In brain slice preparations and whole-cell patch clamp recordings, giant PnC neurons, the well defined reticulospinal neurons, are investigated after using
morphological and electrophysiological features to identify. All recorded PnC neurons showed response to orexin. Orexin induced an inward current on PnC neurons, via postsynaptic orexin 1 and 2 receptors, and decreased the frequency of mEPSC on PnC neurons, through a presynaptic mechanism involving cannabinoid 1 receptor. The mechanism of activation of non-selective cation channels and closure of K+ channels underlying the postsynaptic effect was also revealed. Next, we further found that the amplitude of acoustic evoked EPSC on PnC neurons was decreased consistent with the orexin’s presynaptic effects. Thus, taking advantage of acoustic-evoked startle motor behavior, which has been well known to be PnC-related, the combining post- and pre-synaptic effects of orexin on PnC can be investigated at the behavioral level. As a result, in vivo interference with the orexin system in PnC changed the amplitude and threshold of startle differentially. Considering that orexin deficiency results in cataplexy, a motor deficit characterized by sudden loss of postural muscle tone, and PnC holds a key position in control of muscle tone and posture, the present findings may provide more details concerning the relationship between the absence of orexin and the narcolepsy-cataplexy.

Disclosures: J. Zhang: None. N. Yang: None. Q. Qiao: None.

Poster
476. Opiate, Cytokines, and Other Neuropeptides

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 476.09/A76

Topic: B.01. Neurotransmitters and Signaling Molecules

Title: Projection-target dependent effects of orexin and dynorphin on VTA dopamine neurons

Authors: *C. BAIME1,2, S. L. BORGLAND1;
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Abstract: Ventral tegmental area (VTA) dopamine neurons are a critical part of the neural circuits that underlie reward learning and motivation. Dopamine neurons send dense projections to many brain areas, and recent observations suggest that both the intrinsic properties and the functional output of dopamine neurons can be segregated on the basis of projection target. The output of dopamine neurons can be modulated by input from lateral hypothalamic orexin (also known as hypocretin) neurons. These neurons are critically involved in mediating both arousal and reward-seeking and can release both excitatory orexin peptides and the inhibitory kappa opioid agonist dynorphin. Orexin and dynorphin exert balanced but opposing effects on the firing of VTA dopamine neurons. However, whether orexin and dynorphin can independently
modulate different VTA circuits is unknown. We infused retrograde beads into either the lateral shell of the nucleus accumbens (NAcc) or the basolateral amygdala (BLA) of Pitx3-enhanced green fluorescent protein (Pitx3-eGFP) mice. We then used whole-cell patch clamp electrophysiology to determine the effects of orexin and dynorphin on the firing rate of BLA-projecting or NAcc-projecting VTA dopamine neurons. We found that NAcc-projecting dopamine neurons had greater leak conductance and a greater hyperpolarization-activated inward current (Ih) compared to those that project to the BLA. Moreover, the firing rate of NAcc-projecting dopamine neurons was more likely to be altered by saturating concentrations of orexin and dynorphin than were BLA-projecting dopamine neurons. These results suggest that orexin neurons may independently modulate VTA-NAc and VTA-BLA circuits. Because orexin neurons are a key regulator of dopamine neurons during motivated behavior, these results give further insight into the mechanisms by which orexin neurons regulate the output of the VTA.

Disclosures: C. Baimel: None. S.L. Borgland: None.

Poster

477. GABAA Receptors

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 477.01/A77

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH Grant R01-MH100561

Title: Pubertal expression of α4βδ GABAA receptors reduces discharge of CA1 hippocampus in an epilepsy model

Authors: L. YANG1, H. SHEN1, L. MERLIN1, *S. S. SMITH2; 1Physiol. and Pharmacol., 2Dept Physiol Pharmacol, SUNY Downstate Med. Ctr., Brooklyn, NY

Abstract: More than half of children with epilepsy outgrow their seizures as they mature, yet the underlying mechanism is not yet understood. GABAergic inhibition increases at puberty due to the increased expression of extrasynaptic α4βδ GABAA receptors (GABARs, Shen et al., 2007) on dendritic shafts and spines, when the threshold for triggering an action potential is increased and spiking is decreased. This less excitable state is not observed in animals which do not express α4βδ GABARs, suggesting that these receptors increase hippocampal inhibition during adolescence. Therefore, we tested the role of extrasynaptic α4βδ GABA-A receptors (GABARs) in regulating ictal discharge of CA1 hippocampus in adolescent female mice. To this end, field excitatory post-synaptic potentials (fEPSPs) were recorded from the CA1 stratum radiatum of
hippocampal slices from pre-pubertal (~28-32 PND) and pubertal (~35-44 PND, assessed by vaginal opening) female wild-type or α4/-/- mice at ~30o C using aCSF- filled microelectrodes and an Axoclamp-2A amplifier. Spontaneous bursts were induced by high K+ (8.5 mM) to mimic epileptic discharge (Traynelis and Dingledine, 1988). Cumulative coastline length, a measure of burst amplitude and duration, was assessed, as was the percentage of slices exhibiting ictal discharge. When evoked fEPSPs were recorded in response to increasing stimulation intensities, the input-output curve revealed a 30% reduced response at puberty (P<0.05), compared to pre-puberty, an outcome which was not observed in pubertal α4/-/- hippocampus where responses were similar or up to 10% greater than pre-pubertal values. 8.5 mM K+ induced both ictal and interictal discharge in slices from pre-pubertal animals, with 69.2% exhibiting ictal discharge, a greater proportion than observed in pubertal slices (7.1%, P<0.05). The cumulative coastline length was more than 50% greater in pre-pubertal slices after 100 s exposure to high K+ (2594 ± 469 vs. 1651 ± 221, pubertal, P<0.05). However, recordings from pubertal α4/-/- slices revealed seizure activity (57.1% ictal, 3742 ± 953, coastline length) similar to pre-pubertal, and significantly greater than wild-type (P<0.05). Bath application of 50 nM L-655,708, an inverse agonist at α5-containing GABARs, produced no significant effect on ictal activity in either pre-pubertal or pubertal hippocampus. These data suggest that pubertal expression of hippocampal α4βδ GABARs may selectively reduce pre-existing seizure activity during adolescence.

previous findings suggest that this loss of spines is due to the increased expression of α4βδ GABAA receptors (GABARs) which emerge on dendritic spines during the pubertal period (Shen et al., 2010). α4βδ GABARs generate a shunting inhibition, which impairs activation of NMDA receptors (NMDARs). Although NMDAR activity has been associated with spine maintenance (Alvarez et al., 2007), the precise mechanism for adolescent spine pruning is as yet unknown. Kalirin 7 (Kal7) is a rho-guanine nucleotide exchange factor localized to the postsynaptic density which is necessary for spine stability (Ma et al., 2003). It binds to the NMDAR which can regulate its activation. Thus, we tested whether Kal7 plays a role in adolescent synaptic pruning. We used immunocytochemical techniques to study Kal7 expression in hippocampus and used the Golgi stain to determine spine density changes from Z-stack projection photomicrographs. Spine counts were taken with a Nikon DS-U3 camera mounted on a Nikon Eclipse Ci-L microscope at 100X oil and analyzed with NIS-Elements D 4.40.00 software. Initially, we showed that pubertal synaptic pruning was prevented by increasing NMDAR expression (MK-801, 0.25 mg/kg, i.p., 5 d). Conversely, blocking NMDARs (memantine, Mem, 10 mg/kg, i.p., 5 d) in the pubertal α4-/- mouse triggered synaptic pruning, resulting in a 50-60% decrease in spines (P<0.05), suggesting a role for NMDARs in spine maintenance during adolescence. Hippocampal expression of Kal7 decreased by 30% at puberty (P<0.05), an effect prevented by α4 knock-out, implicating α4βδ GABARs in eliciting the pubertal decrease in its expression. In vivo blockade of NMDAR with Mem reduced hippocampal Kal7 expression by half (P<0.05), suggesting that NMDAR activation maintains Kal7 expression. As predicted, Kal7-/- mice exhibited reduced spine density in the CA1 hippocampus during puberty compared to wild-type mice (spines/ 20 µm: 16 ± 1.5, WT, vs. 9.4 ± 0.8, Kal7-/-, P<0.05). These data suggest that NMDARs increase Kal7 expression, which is necessary for spine stability. Impairment of NMDAR activation via α4βδ GABARs during puberty reduces Kal7 expression, reducing spine stability, thereby reducing spine density. These data suggest that α4βδ GABARs play a role in adolescent synaptic pruning by impairing NMDA receptor activation which reduces Kal7 expression.

Disclosures: J. Parato: None. S. Afroz: None. S. Smith: None.

Poster

477. GABAA Receptors

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 477.03/A79

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH T32 Training Grant
Title: Perinatal hypoxia increases GABA release in the auditory brainstem during early development

Authors: *M. A. HAROON, S. Y. LEE, J. XU, C. GRANADOS, J. H. KIM; UTHSCSA, San Antonio, TX

Abstract: Perinatal hypoxia is a causative factor in many disorders, including neurosensory disorders, mental retardation and autism. One mechanism by which hypoxia/ischemia causes damage is an increase in glutamate-mediated excitotoxicity. Recent research has shown that neurons in brain slices can counteract the effects of excitotoxicity by increasing spontaneous GABA release (DeFazio et al, 2009). However, it is unknown if this applies to live animals that suffer perinatal hypoxic insults. We investigated medial nucleus of the trapezoid body (MNTB) neurons from hypoxia-exposed rats to find if this would increase GABA release as well as glutamate release, whether this effect is dependent on developmental stage, and if this would have a neuroprotective effect. In the postnatal first week (P5-P8), we found an increase in GABA_A receptor-mediated spontaneous inhibitory postsynaptic currents (sIPSCs) and a decrease in the amplitude of evoked excitatory postsynaptic currents (EPSCs) in the perinatal hypoxia group. These results were confirmed by in vitro oxygen-glucose deprivation experiments on P7 brain slices. Immunohistochemistry demonstrated an increase in the co-localization of vesicular GABA transporter (VGAT) and vesicular glutamate transporter 1 (VGluT1) in the hypoxia group (P7) indicating an increase of GABAergic innervation to presynaptic calyx terminals as well as postsynaptic MNTB neurons. However, these changes are developmentally dependent, as GABA signaling returned to normal by P13-P17 in the perinatal hypoxia group. These results indicate immature MNTB neurons respond to hypoxic insult by increasing spontaneous GABA release, which may trigger the potential neuroprotective mechanisms against hypoxic injury in the neonatal brain.


Poster

477. GABAA Receptors

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 477.04/A80

Topic: B.02. Ligand-Gated Ion Channels

Support: NINDS Grant NS051590 08
Title: Rescue of a mutant GABA(A) receptor subunit by functional complementation

Authors: *L. G. JACKSON, R. L. MACDONALD, C. HERNANDEZ, N. HU;
Neurol., Vanderbilt Univ., Nashville, TN

Abstract: GABA_A receptors are the primary mediators of inhibition in the brain. Mutations in GABA_A receptor subunits have been identified in patients with genetic generalized epilepsy disorders (GGEs). These mutations have can affect the assembly, trafficking, ligand binding, and gating of the receptors, which can impair neuronal inhibition. A mutation identified in a family with generalized epilepsy with febrile seizures plus (GEFS+), including the severe epileptic encephalopathy Dravet syndrome, introduces a premature stop codon in the γ2 subunit, which results in the truncated subunit γ2(Q390X). This mutation reduces cell-surface expression of γ2-containing GABA_A receptors in a dominant-negative fashion. In an effort to rescue the surface expression of functional receptors, γ2(Q390X)-containing GABA_A receptors were coexpressed in a heterologous expression system with the γ2 C-terminal domain beginning at residue Q390 (CTD-390), and the surface expression of GABA_A receptor subunits was assessed by flow cytometry. Surface expression of γ2 and γ2(Q390X) increased when coexpressed with CTD-390. In addition, electrophysiological studies indicate that mutant-containing receptors exhibit kinetic properties similar to wild type γ2-containing GABA_A receptors. These results suggest that the dominant-negative effects of γ2(Q390X) can be overcome by independent expression of the missing C-terminal domain, and that rescue of the surface expression of γ2(Q390X)-containing GABA_A receptors could increase inhibitory neurotransmission in vivo.


Poster

477. GABAA Receptors

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 477.05/A81

Topic: B.02. Ligand-Gated Ion Channels

Support: Swiss National Science Foundation grant 315230_156929/1

M.C.M. is a recipient of a fellowship (Beca Chile Postdoctorado from CONICYT, Ministerio de Educacion, Chile)

Title: Understanding the low affinity actions of benzodiazepines on GABAA receptors
Authors: *M. C. MALDIFASSI, R. BAUR, E. SIGEL;* 
Univ. of Bern, Bern, Switzerland

**Abstract:** Benzodiazepines interact with $\alpha_x\beta_2\gamma_2$ ($x = 1, 2, 3$ and 5) GABA$_A$ receptors modulating their function through binding to a high affinity site located at the $\alpha/\gamma$ interface. Together with the existence of this high affinity binding site (site 1), there is evidence for the existence of two low affinity sites for benzodiazepines. An inhibitory site (site 2) has been postulated to locate to the $\alpha/\beta$ interface. An additional modulatory site (site 3) is presumably located in the membrane and has been shown to be abolished upon combined mutation of residue $\beta_2265$ and homologous residues in other subunits ($\alpha_1S269I$, $\beta_2N265I$, $\gamma_2S280I$). With the aim to further describe sites 2 and 3, we performed a detailed study of the properties of receptors carrying a single mutation, and also concatenated receptors carrying them. The consequences of the mutations on diazepam and flurazepam induced GABA modulation were studied, thus site 2 could be located more precisely. We studied also the effect of individual sites 3 mutations ($\alpha_1S269I$, $\beta_2N265I$ and $\gamma_2S280I$). While the concentration dependence of flurazepam was bell-shaped and independent of the three mutations affecting sites 3, that of diazepam was sensitive to the mutations. While mutation of $\alpha_1$ and $\beta_2$ subunits abolished potentiation via sites 3, the mutation in the $\gamma_2$ subunit stimulated it. Thus, a site 3 for diazepam is putatively located to each subunit. In contrast, flurazepam fails to act at sites 3. CGS9895 has been postulated to act at the extracellular portion of the $\alpha/\beta$ interface. Both, the above mutations in the $\alpha_1$ and the $\beta_2$ subunit abolished potentiation by CGS9895, while the mutation in the $\gamma_2$ subunit was without effect. If the membrane part of the receptor allosterically signals to the extracellular part of the $\alpha/\beta$ interface, it has to be postulated that membrane portions of both $\alpha$ and $\beta$ subunits do this. Alternatively CGS9895 could act at site 3. The presence of the $\alpha_4$ subunit in the $\alpha_4\beta_2\gamma_2$ receptor is considered to cause the receptor to be insensitive to diazepam. We observed that higher concentrations of diazepam potentiated GABA gated currents, indicating the presence of site 3 in this receptor.

**Disclosures:** M.C. Maldifassi: None. R. Baur: None. E. Sigel: None.

**Poster**

**477. GABAA Receptors**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 477.06/A82

**Topic:** B.02. Ligand-Gated Ion Channels

**Support:** NIH Grant N0034774

AES Fellowship SPO-117132
Title: A novel role for autophagy in endogenous GABA-A receptor modulation

Authors: *A. M. ABRAMIAN, J. S. SOROKIN, C. D. MAKINSON, J. R. HUGUENARD; Neurol., Stanford Univ., Palo Alto, CA

Abstract: Abnormally synchronous neuronal activity is a common feature of seizures and current pharmacological management of epilepsy works at least in part through suppression of neuronal firing. It has been increasingly recognized that astrocytes, in addition to their well-characterized role as supportive elements, have active roles in circuit development and regulating synaptic transmission. We have recently shown that an endogenously occurring benzodiazepine site ligand (endozepine), arising from the Diazepam Binding Inhibitor (DBI) protein, enhances GABAergic inhibitory synaptic transmission in the thalamic reticular nucleus (nRt), an essential region for regulation of generalized absence seizures. Mice lacking the DBI gene or the benzodiazepine (BZ) binding site on GABAA receptors in nRt neurons showed spontaneous absence seizures and increased susceptibility to chemoconvulsants. Astrocytes express the highest levels of DBI and utilize an unconventional autophagic pathway to secrete endozepines. In the present study, we utilize whole cell recordings in thalamic slices at near physiological temperature (32°C) along with pharmacology to acutely modulate the level of autophagy and determine if there is any modulation of GABAA receptor activity in nRt neurons. Consistent with an endozepine effect, locally perfused rapamycin (200 nM), which promotes autophagy, induces within minutes a significant increase in the half-width of spontaneous inhibitory postsynaptic currents (sIPSCs) in the nRt neurons (vehicle control: 6.80 +/- 0.5 ms, n = 6; 200 nM rapamycin: 10.8 +/- 1.0 ms, n = 6). This effect is blocked by flumazenil (FLZ), a silent modulator, or BZ site antagonist, that allosterically binds to the BZ site. These results demonstrate dynamically regulated autophagic secretion of an endozepine with GABAA receptor positive allosteric modulation (PAM) effects. Selective release of endozepines from astrocytes, and the subsequent reduction of nRt activity, may prove to have powerful seizure suppressing effects.


Poster

477. GABAA Receptors

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 477.07/A83

Topic: B.02. Ligand-Gated Ion Channels
Title: Cysteine scanning of loop G residues in GABAA receptors

Authors: *D. BAPTISTA-HON, T. G. HALES;
Inst. of Academic Anaesthesia, Dundee, United Kingdom

Abstract: γ-aminobutyric acid type A (GABA_A) receptor is a pentameric ligand gated ion channel which mediates inhibitory neurotransmission in the brain. GABA binding to the orthosteric site, located at the interface between adjacent β (positive interface) and α (negative interface) subunits, activates the integral Cl^- channel. Residues responsible for agonist binding in the orthosteric site arise from 6 non-continuous loops: A, B and C from the β subunit, and D, E and F from the α subunit. High-resolution glutamate-bound crystallographic structures of the *C. elegans* glutamate activated chloride channel (GluCl), as well as acetylcholine-bound *E. Chrysanthemi* ligand gated ion channel (ELIC) infer residues on the β1 strand on the complimentary interface to stabilise bound ligand. Residues in the β1 strand are outwith any of the 6 known loops and therefore putatively named loop G. Arg37 in GluCl, and Phe19 in ELIC extends into the orthosteric binding site and stabilises bound glutamate, and acetylcholine, respectively. The homologous residues on the GABA_A α1 subunit are Thr47 and Phe45, respectively. These residues, and others in the β1 strand, have not been implicated in agonist activity in mammalian Cys-loop receptors. We substituted Thr47 to Cys in the GABA_A α1 subunit and transfected these, along with β2 and γ2 subunits into HEK-293 cells. Application of varying concentrations of GABA to voltage-clamped HEK-293 cells expressing α1(T47C)β2γ2 receptors reveal a reduced apparent potency (EC_{50} = 170 µM), when compared with α1β2γ2 receptors (EC_{50} = 18 µM). Application of MTSEA (10 mM) significantly reduced the size of the current evoked by a maximally efficacious concentration of GABA at α1(T47C)β2γ2 receptors, but not α1β2γ2 receptors, suggesting that Cys47 is accessible for modification. Modification was prevented if MTSEA was applied together with a maximally efficacious concentration (10 mM) of GABA at α1(T47C)β2γ2 receptors, suggesting that GABA can protect Cys47 from modification. Our preliminary data indicate that Thr47 in the putative loop G within the β1 strand influences the apparent potency of GABA. SCAM analysis reveals that this residue may be part of a water-accessible pocket where GABA binds. To characterise the contribution of other β1 strand residues to GABA_A receptor function, we will investigate the effect of Cys substitution at other positions on the β1 strand.

Disclosures: D. Baptista-Hon: None. T.G. Hales: None.

Poster

477. GABAA Receptors

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM
Title: Somatostatin gabaergic interneurons regulate gastric circuit activity within the dorsal vagal complex

Authors: *A. LEWIN, S. VICINI, R. GILLIS, N. SAHIBZADA; Pharmacol. and Physiol., Georgetown Univ. Med. Ctr., Washington, DC

Abstract: The dorsal motor complex (DVC) regulates parasympathetic control of the stomach. Within the DVC there are three nuclei of importance: the area postrema, the nucleus tractus solitarius (NTS), and the dorsal motor nucleus of the vagus (DMV). The final parasympathetic output from the DMV projects to distinct regions of the stomach, each with specific functions. Of particular importance for our studies is the projection to the gastric antrum, which controls gastric motility. Previous studies have focused on the regulation of the NTS and the DMV, both of which are under tonic GABAergic control. *In vivo* microinjection of the GABAA blocker bicuculline into the NTS or DMV has profound but differential effects on motility. What has yet to be determined is the source of this GABAergic inhibition. The DVC contains a large population of somatostatin positive GABAergic interneurons (Sst-GABA) interspersed among all three nuclei. Sst-GABA interneurons have been shown to play integral roles in regulating the activity of other brain regions. Our studies focus on establishing Sst-GABA neurons as the primary source of GABAergic regulation in both the NTS and the DMV. We used transgenic mice expressing either an excitatory opsin (channelrhodopsin, CHR2) or an inhibitory opsin (archaerhodopsin, Arch T) under control of the Sst-cre driver. This allows specific excitation or inhibition of Sst-GABA neurons. Additionally, either the polysynaptic tracer pseudo rabies virus (PRV-152) or the monosynaptic tracer DiI was injected into the gastric antrum, which allowed visualization of neurons specifically associated with control of the gastric antrum. Electrophysiological recordings were then made from PRV-152 labeled neurons or DiI labeled neurons. Recorded cells were separated for analysis based on whether or not a cell exhibited a direct opsin current, allowing for groups of Sst-GABA neurons and non Sst-GABA neurons. CHR2 stimulation caused an inhibition of action potentials and an excitation of inhibitory post synaptic currents (IPSCs) in non Sst-GABA neurons located in the NTS or DMV. Interestingly, CHR2 stimulation also elicited an increase in IPSCs in Sst-GABA neurons both in the NTS and the DMV. ArchT stimulation resulted in an attenuation of post synaptic currents (PSCs) in both non Sst-GABA neurons and Sst-GABA neurons in the NTS and DMV. These studies indicate that output neurons in the DMV and potential output neurons in the NTS are under tonic inhibitory control from Sst-GABA neurons. Additionally, this data suggests that Sst-GABA neurons form a complex interactive regulatory network within both nuclei.

Title: Characterizing the guanidine compound interaction site in the GABA-A rho1 receptor

Authors: *H. D. SNELL*¹, E. B. GONZALES¹²;
¹Univ. of North Texas Hlth. Sci. Ctr., Fort Worth, TX; ²Inst. for Aging and Alzheimer's Dis. Res., Fort Worth, TX

Abstract: GABAC, or GABAA rho, is a subclass of GABAA receptors composed entirely of rho (ρ) subunits located on the axonal terminal of retinal bipolar cells. GABAA-rho exhibits unique properties, such as insensitivity to select antagonists of the heteromeric GABAA receptors. A group of ligands, which possess a guanidine group, have been shown to influence GABAA receptors. This includes the acid sensing ion channel (ASIC) ligand, amiloride, most commonly used to treat hypertension, which is also most prevalent in those 60 years of age and older. Point mutations revealed the 15' mutation (I15'N) completely removed the stimulatory activity of amiloride, while the 19' mutation (N19'D) reversed the potentiation, resulting in inhibition. Thus we believe these residues are integral in the mechanism of action these guanidino compounds with the GABA-A rho1 receptor. Our next step was to elucidate the orientation of the compounds, indicating if it is indeed the guanidine group that interacts with the receptor. We utilized 3 amiloride derivatives, 5-(N,N-Hexamethylene)amiloride (HMA), benzamil, and phenamil. Both benzamil and phenamil alter the guanidine group, while HMA has a ring on the opposite end. Whole cell patch electrophysiology of wild type GABA-A rho1 shows that both benzamil and phenamil elicit no change in GABA induced current, while HMA has increased potency compared to amiloride and exhibits similar potentiation of current. Our findings suggest that the guanidino group on amiloride is interacting with the GABA-A rho1 receptor to potentiate activity. These results reveal a novel compound interaction with the GABA-A rho1 receptor and could aid in producing new pharmacological compounds containing this guanidino group.
backbone. This also suggests contrary to previous studies, tamiloride could exacerbate retinal hypoxic disorders, such as diabetic retinopathy, prevalent in the aged population.

Disclosures: H.D. Snell: None. E.B. Gonzales: None.

Poster

477. GABAA Receptors

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 477.10/A86

Topic: B.02. Ligand-Gated Ion Channels

Support: Sage Therapeutics, Inc.,
Simons Foundation Grant 206026
NIH–NIMH Grant MH097446
NIH–NINDS Grants NS051195, NS056359, and NS081735

Title: SGE-516, a novel neuroactive steroid mediates an increase in the efficacy of tonic inhibition via an increase in the trafficking of extrasynaptic GABAA Receptors

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Abstract: In the dentate gyrus, neocortex, striatum and the thalamus tonic inhibition is largely dependent on GABAARs composed of α 4, β 2/3, and δ subunits. These receptors are particularly sensitive to allosteric modulation by neuroactive steroids (NAS). Modifications in tonic inhibition and fluctuations in endogenous levels of NAS are accepted to play critical role in epileptogenesis and numerous neuropsychiatric disorders. While the ability of NAS to allosterically enhance GABAAR gating is well established, we have recently shown that NAS can also exert persistent effects on the efficacy of tonic inhibition by increasing PKC-mediated phosphorylation of the α 4 subunit. This in turn increases the membrane expression and boosts tonic inhibition. We have examined both naturally occurring NAS as well as a novel synthetic NAS (SGE-516) that display improved physicochemical and pharmacokinetic properties. SGE-516 is a novel synthetic neuroactive steroid. We assessed the ability of SGE-516 to potentiate GABA currents in recombinant GABAA receptors. SGE-516 potentiated GABA currents at α 1
β2γ2 subunits with an EC50 of 61nM and Emax of 219%. SGE-516 was also effective at prototypical extrasynaptic GABA receptors, potentiating GABA currents at recombinant α4β3δ subunits with an EC50 of 227nM and Emax of 646%. Also, we have examined SGE-516 in dentate gyrus granule cells (DGGCs) from hippocampal slices. A 10min exposure to 100nM SGE-516 followed by ≥ 30 min wash induced a ∼ 2 fold increase in tonic current. This increase was prevented by inhibiting PKC with GF 109203X. To examine the allosteric effects of SGE-516, we treated the slices with 100 nM SGE-516 acutely and observed a small potentiation of tonic current in DGGCs of hippocampal slices. Our studies have shown that in addition to the allosteric modulation of GABAARs, a general mechanism for NAS to increase the efficacy of tonic inhibition via an increase in extra-synaptic GABAAR trafficking through a PKC mediated mechanism.


Poster

477. GABAA Receptors

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 477.11/A87

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH RO1 NS 33300

Title: Unraveling the epileptogenic properties of GABAA receptor subunit interfaces


Abstract: A number of mutations in GABA_A receptor subunit genes (GABRs) that impair GABA_A receptor function by causing gating or trafficking deficiencies have been described in patients with genetic epilepsies. Recently, de novo mutations in GABRB3, N110D, D120N, E180G, Y302C, and GABRB1, F246S, were associated with the severe epileptic encephalopathies Lennox-Gastaut syndrome (LGS) and infantile spasms (IS) by the Epi4K consortium (Allen et al., 2013). Up to now only three mutations in GABRB3, P11S, S15F and G32R, have been associated with childhood absence epilepsy (CAE), a mild epilepsy characterized by typical absence seizures. We found that that both LGS- and IS-associated GABRB mutations
significantly impaired \( \text{GABA}_A \) receptor gating, which seemed to be structurally correlated with the location of the mutation in the receptor. Considering the severity of the epilepsy phenotype exhibited by patients with LGS and IS, we found that LGS-associated \( \text{GABRB3}(D120N, E180G, Y302C) \) mutations caused larger effects on \( \text{GABA}_A \) receptor function than the IS-associated \( \text{GABRB3}(N110D) \) and \( \text{GABRB1}(F246S) \) mutations, and the CAE-associated \( \text{GABRB3}(P11S, S15F, G32R) \) mutations. While LGS-associated \( \text{GABRB3}(D120N, E180G, Y302C) \) mutations are in the \( \beta^+\alpha^- \) subunit interface, which is directly coupled with the ligand-binding-channel-coupling pathway of the receptor, IS-associated \( \text{GABRB3}(N110D) \) and \( \text{GABRB1}(F246S) \) mutations are in the \( \alpha^+\beta^-\gamma^+\beta^- \) interfaces that are indirectly coupled by subunit rearrangements throughout the \( \beta^- \)-sheets/\( \alpha^- \)-helices of the receptor. Interestingly, the \( \text{GABRB3}(G32R) \) mutation associated with the mild epilepsy, CAE, is located at the \( \beta^- \) interface in the distal N-terminal \( \alpha^-1 \)-helix of the receptor where it was predicted to disrupt local salt bridges at the \( \gamma^+\beta^- \) interface important for assembly and function of \( \text{GABA}_A \) receptors containing \( \beta^-3 \) subunits. Thus, it seems that the presence of mutations at the \( \beta^+\alpha^- \) interface might cause major structural rearrangements of crucial domains important for translating ligand binding to channel gating of the \( \text{GABA}_A \) receptor and could be associated with the severity of the channel dysfunction and the epilepsy phenotype. This work was supported by the NIH RO1 NS 33300 grant to RLM.

**Disclosures:** C.C. Hernandez: None. V.C. Satpute: None. R.L. Macdonald: None.

**Poster**

**477. GABA\(_A\) Receptors**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#: 477.12/A88**

**Topic:** B.02. Ligand-Gated Ion Channels

**Support:** European Research Council Advanced Grant

Academy of Finland

Academy of Finland (ERA-Net NEURON II CIPRESS)

The Sigrid Jusélius Foundation

The Jane and Aatos Erkko Foundation

**Title:** Impermeant anions, fixed charges, and the driving force of GABA\(_A\)-mediated Cl-currents
Authors: *J. T. VOIPIO*, K. KAILA;
1Dept. of Biosciences, Univ. of Helsinki, Helsinki, Finland; 2Dept. of Biosci. and Neurosci. Center, Univ. of Helsinki, Helsinki, Finland

Abstract: The key role of the cation-chloride cotransporters (CCCs) KCC2 and NKCC1 in neuronal Cl- regulation and in setting the driving force (DF) of GABAergic receptor-mediated Cl-currents has been demonstrated using electrophysiological, pharmacological and genetic approaches (1). Contrary to compelling evidence, it has been recently suggested that the DF and polarity of GABAergic currents are set by electrostatic (Donnan) effects by immobile anions acting on local Cl- concentrations and thereupon on CCCs (2,3). However, transmembrane ion currents dissipate energy, and passive immobile anions neither do work nor consume energy. Electrostatic effects can alter the concentrations of mobile ions in aqueous microdomains where binding of ions to CCCs takes place, but this has no effect on the free energy of ion transport which depends on differences between intracellular and extracellular bulk phases (1,4). The Donnan mechanism at a Cl- permeable membrane acts to establish an equilibrium distribution of Cl-, not a DF for Cl- current. If present, intracellular spatial gradients of immobile charges generate parallel cytosolic electrical potential gradients along which freely mobile ions equilibrate. This will result in identical shifts of membrane potential and the equilibrium potential of the ions, and there will be no changes in the transmembrane ionic DFs. Thus, Donnan mechanisms across a plasma membrane or along cytosol (2,3) cannot account for observations such as depolarizing and hyperpolarizing GABAergic Cl- currents in the axon initial segment and dendrites, respectively (5,6). Basic thermodynamic principles rule out the possibility of using microdomains of restricted water (3) as a source of energy for Cl- transport, and there is no evidence supporting the idea (2,3) that KCC2 expressed in CNS neurons transports ~500 water molecules along with one K+ and Cl- ion. We conclude that the ideas (2,3) on the role of immobile anions and restricted water in determining the polarity of channel-mediated Cl- currents conflict with thermodynamics and with the overwhelming amount of evidence demonstrating the role of CCCs in neuronal Cl- regulation. References 1. Kaila et al. 2014, Nat Rev Neurosci. 15: 637–54 2. Glykys et al. 2014 Science 343:670-5 3. Delpierre and Staley 2014 J Physiol. 592: 4099-114 4. Voipio et al 2014. Science 345: 1130 5. Szabadies et al. 2006. Science 311: 233-5 6. Khirug et al. 2008. J Neurosci. 28: 4635-9

Disclosures: J.T. Voipio: None. K. Kaila: None.

Poster
477. GABAA Receptors

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM
Title: Novel benzodiazepines selectively binding to α5-containing GABAA receptors

Authors: *P. SCHOLZE*¹, R. PUTHENKALAM¹, M. TREVEN¹, J. RAMERSTORFER¹, M. M. POE², K. R. METHUKU², G. LI², W. SIEGHART¹, J. M. COOK², M. ERNST¹;
¹Med. Univ. Vienna, Vienna, Austria; ²Univ. of Wisconsin-Milwaukee, Milwaukee, WI

Abstract: In the mammalian nervous system, GABAA receptors exist in an enormous diversity of subtypes. Six α, three β, three γ, the δ, ε, θ, π, and three ρ subunits can, and most of them actually do, assemble into a multitude of different homo- or hetero-pentameric receptors, each of them displaying unique pharmacological properties. GABAA receptors are targeted by various clinically relevant drugs such as for example the widely prescribed benzodiazepines, which can exert many different effects, including anxiolytic, sedative, hypnotic, amnesic, anticonvulsant, and muscle relaxant actions. The different benzodiazepine-induced behavioral responses seem to be predominantly mediated by GABAA receptors subtypes containing specific α subunits. This precipitated a search for novel ligands that discriminate between different α-containing receptors. Some α5-prefering (selective) compounds have already been identified, such as the chiral imidazobenzodiazepine SH-053-2’-F-R-CH3 (Gallos et al., 2015) and other structurally similar compounds (Puthenkalam et al, manuscript in preparation). The most selective compound among them binds to α5 with approximately 60-fold higher affinity compared to α1. Binding and efficacy selectivity however do not necessarily correlate. Some ligands exist, which bind to several subtypes with comparable affinity, but are “silent” or very weak allosteric modulators, at some subtypes, while acting as positive or negative allosteric modulators at other subtypes. The molecular determinants that lead to functional or binding selectivity on the GABAA-receptor α5-subunit are however still completely unclear. Aim of the current study is to compare the binding affinities and electrophysiological efficacies of several different benzodiazepines, in order to correlate structure with affinity or efficacy. In order to identify amino-acid residues on the α5 subunit, which lead to selective benzodiazepine binding and/or selective modulation of GABA induced currents, mutated subunits have been constructed. Those will be expressed in heterologous expression systems and tested using radioligand binding assays and oocyte electrophysiology.


Flumazenil alters GABAergic neurotransmission via direct channel modulation and changes in post-synaptic protein expression

Authors: *I. A. SPEIGEL*¹, Q. XU¹, V. CIAVATTA¹, A. JENKINS¹, P. S. GARCÍA¹,²; ¹Anesthesiol., Emory Univ., Atlanta, GA; ²Res. Div., Atlanta VA Med. Ctr., Atlanta, GA

Abstract: Flumazenil has recently been demonstrated to relieve excessive sleepiness in patients who exhibit hypersomnia due to increased GABA_A receptor function. The underlying mechanism for this treatment is still poorly understood. Flumazenil is best characterized as a GABA_A receptor benzodiazepine binding-site antagonist, but behavioral measures in animals suggest a more complicated pharmacological profile. To investigate the action of flumazenil on GABAergic neurotransmission, we tested its effects on GABA_A receptor channel function and related protein expression. Electrophysiological recordings in a HEK293 cell-based heterologous expression system are consistent with flumazenil being a competitive antagonist of GABA at synaptic-type (α1β2γ2) receptors. Flumazenil depresses chloride currents evoked by high concentrations of GABA, with little effect on currents evoked by low concentrations of GABA. Intrinsically, flumazenil shows weak agonist activity and evokes chloride currents in a sigmoidal dose-response relationship. In primary cortical neuronal cultures, protein measures show that 24 hour exposure to low-dose flumazenil increased β2 GABA_A receptor subunit expression, while high doses had no effect. In contrast, flumazenil did not alter extrasynaptic receptor α4 subunit, GAD67, and neuroapoptotic marker expression levels. A cell-titer blue assay of viability also suggests no overt evidence for altered rates of cell death. These results suggest that in addition to direct channel modulation, flumazenil is altering GABAergic neurotransmission by evoking an increase in synaptic-type receptor number. Preliminarily, we suspect that the low-dose flumazenil mediated increase in β2 subunit protein expression is a compensatory response to antagonized GABAergic neurotransmission and increased neural culture excitability. As a therapy, flumazenil can pharmacologically enhance GABA-ergic transmission and therefore may not be an effective treatment for general sleepiness in patients with normal GABA activity.
Histone deacetylase inhibitors prevent chronic ethanol-induced adaptations in GABAA receptor subunit expression in cortical neurons

**Authors:** *J. P. BOHNSACK*¹, A. L. MORROW²;  
¹Pharmacol., ²Pharmacol. and Psychiatry, Univ. of North Carolina At Chapel Hill, Chapel Hill, NC

**Abstract:** There is increasing evidence that chronic ethanol exposure induces chromatin remodeling. Treatment with histone deacetylase inhibitors can prevent the behavioral and molecular effects of chronic ethanol exposure and withdrawal. Chronic ethanol exposure alters GABAA-R subunit expression and function and contributes to the development of alcohol dependence and withdrawal symptoms. Thus, we investigated whether histone deacetylase inhibitors could prevent chronic ethanol-induced changes in GABAA-R subunit gene and protein expression. We have previously shown that ethanol exposure of cortical cultured neurons recapitulates the changes in GABAAα1 and α4 subunit expression in cortex of ethanol dependent Sprague Dawley rats. Cortical neurons from PD0-1 Sprague Dawley rat pups were cultured for 18 days. Ethanol (50 mM) was added to the media for 4 hrs in the presence and absence of the HDAC inhibitors SAHA (300 µM) or TSA (500 nM) to examine changes in GABAAα1 and α4 subunit expression measured by qPCR and western blot analysis. Ethanol decreased GABAAα1 gene expression and increased GABAAα4 gene expression which was prevented by SAHA co-exposure. TSA also prevented changes in ethanol-induced decrease in α1 gene expression. We next analyzed changes in membrane protein expression using western blot and found that SAHA prevents the ethanol-induced decrease in GABAAα1 subunit and the increase in α4 subunit expression. Neurons treated with TSA also prevented decreases in α1 subunit protein expression. TSA nor SAHA treatment alone had any significant effect on either α1 or α4 subunit gene and protein expression. Next, we addressed the possibility that HDAC inhibition may have an effect on receptor trafficking. We found that SAHA prevents ethanol-induced decreases in GABAAα1
subunit surface expression. Finally, since PKA plays a role in chromatin remodeling we wanted to determine if PKA was involved in facilitating SAHA’s ability to prevent ethanol-induced changes in GABA$_{A}$ subunit expression. We exposed cortical neurons to the PKA inhibitor RpCAMPs (50 µM) along with SAHA and ethanol. RpCAMPs exposure did not alter the effect of ethanol + SAHA on either $\alpha_1$ or $\alpha_4$ subunit gene expression compared to SAHA and ethanol treated neurons. This suggests that PKA is not required for SAHA to prevent ethanol-induced changes in GABA$_{A}$ subunit gene expression. Our results indicate that treatment with HDAC inhibitors reverses the effects of chronic alcohol exposure on GABA$_{A}$-R subunit expression. This work represents an additional line of evidence suggesting the use of histone deacetylases inhibitors for the treatment of alcohol use disorders.

Disclosures: J.P. Bohnsack: None. A.L. Morrow: None.

Poster

477. GABA A Receptors

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 477.16/A92

Topic: B.02. Ligand-Gated Ion Channels


Title: Astrocytes selectively regulate the expression of different GABA-A receptor subunits in cortical neurons

Authors: *A. BERRETTA$^{1,2}$, A. N. CLARKSON$^{1,2,3}$;
$^{1}$Anat., Univ. of Otago, Dunedin, New Zealand; $^{2}$Brain Hlth. Res. Ctr., Dunedin, New Zealand;
$^{3}$Fac. of Pharm., The Univ. of Sydney, Sydney, Australia

Abstract: Introduction GABA-A receptors are heteropentameric subunit complexes and the different subunit compositions influence the receptor's agonist affinity, chance of opening, conductance, and cellular localization (extrasynaptic or synaptic). Astrocytes play a crucial role in both the formation and function of synapses and the effects of astrocyte-secreted factors is well documented for glutamate receptors. However, the astrocytic influence on GABA-A receptors has been only partially investigated and the attention was only focused on the synaptic GABA-A subunits. Objective The aim of this study was to investigate whether factors released from astrocytes can influence the neuronal expression of GABA-A receptor subunits during neurodevelopment and after brain injury. Methods This study is based on an in vitro system that is constituted by two different primary cultures of pure neurons and pure astrocytes from cortex.
Astrocytes were cultured using different conditions. In injury condition, astrocytes were mechanically stretched to render them reactive. The astrocyte-conditioned media were collected and used to treat neuronal cultures. Changes in expression of various GABA-A receptor subunits were assessed using real-time PCR. Results Analysis of mRNA expression for the GABA-A alpha-4 (α4) subunit showed a significant and time-dependent decrease in neurons at 5 and 9 days after 24h-treatment with control astrocyte media. In addition, decrease in α4-subunit expression was more pronounced when neurons were treated with media collected from injured astrocytes. Surprisingly, analysis of delta (δ) subunit, an extrasynaptic subunit that preferentially coassemble with α4, revealed an increase in expression in neurons exposed to injured astrocyte media. Further, we found no significant differences in the expression β2 and γ2 subunits after exposure to media collected from either control or injured astrocytes. Conclusions α4 and δ subunit expression shows that astrocytes selectively regulate neuronal GABA-A subunits. The effects observed with injured astrocytes suggest that brain trauma or stroke might be associated with an astrocyte-mediated change of GABA-A expression. Future studies are planned to assess the location of these subunits around the surface of the neurons and the impact that this has on drug efficacy and function.

Disclosures: A. Berretta: None. A.N. Clarkson: None.
the previously reported DPP derivatives, WSS-4a induced significant amounts of current with a high affinity interaction. At α1β2γ2s receptors, the dose response curve fit a one site model. The EC50 was 0.011 nM with a maximal induced current of 69% of maximal (1 mM) GABA. The response seems similar for α1β2γ. However, the addition of the large butyl group did subtly affect the response. The Hill slope was less than one, in contrast to the difluoro- (WSS-2) derivative; and at the α1β2γ2s receptors, the decline in induced current seen with higher concentrations of WSS-2 was absent with WSS-4a. Therefore, the addition of the butyl group did have some effect. Questioning why these DPP derivatives have strong GABA_A agonist activity, we used the jmol program to look for structural similarities between DPP, other antihistamines, GABA, and the piperidine GABA agonist THIP. We found a potential area of similarity based near the amine group and adjacent hydrocarbon backbone of these compounds. By targeting that area, and perhaps the location of the butyl group, we may be able to design more specific high affinity GABA ligands or antihistamines.


Poster

477. GABAA Receptors

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 477.18/A94

Topic: B.02. Ligand-Gated Ion Channels

Title: Complex changes in GABAA receptor gating caused by mutations associated with alcohol preference

Authors: S. GULBINAITE, D. BAPTISTA-HON, F. ROBERTSON, *T. G. HALES;
Univ. of Dundee, Dundee, United Kingdom

Abstract: Spontaneous gating in the hippocampus and dentate gyrus contributes to persistent neuronal inhibition mediated by γ-aminobutyric acid type A (GABA_A) receptors. Bicuculline and picrotoxin block spontaneous GABA_A receptor gating, while gabazine is ineffective. Single point mutations leading to P228H and L285R amino acid substitutions in the GABA_A β1 subunit, associated with alcohol preference in mice cause increased spontaneous gating. To investigate whether these substitutions also cause spontaneous gating when introduced into α1 or other β subunits we expressed each within α1βxγ2 receptors in HEK293 cells for investigation by voltage-clamp electrophysiology. The level of spontaneous current (Ispont) blocked by picrotoxin was expressed as a percentage of the total current mediated by GABA_A receptors (Itotal) in whole-cell recordings. Both P228H and L285R substitutions increased Ispont (to 20-60% of Itotal) in all β
subunit backgrounds. GABA$_{\alpha_1}\beta_1$(P228H/L285R)$_{\gamma_2}$ receptors exhibited current overshoot following the application of either picrotoxin or GABA. Determination of the Cl$^-$ equilibrium potential using voltage-ramp experiments revealed that $I_{\text{spont}}$ depleted intracellular Cl$^-$ and recovery from depletion contributed to the current overshoot. However, $I_{\text{spont}}$ overshoot was also evident in outside-out patch recordings where there was no Cl$^-$ depletion. This may indicate that $I_{\text{spont}}$ initiates desensitization, which is alleviated by picrotoxin. Preliminary experiments examining the kinetics of GABA-evoked currents mediated by $\alpha_1\beta_1$(P228H/L285R)$_{\gamma_2}$ reveal altered desensitization and deactivation kinetics. These findings have important implications regarding mechanisms of inhibitory neurotransmitter receptor gating and the effects of pathological channelopathies on their function.

Disclosures: S. Gulbinaitė: None. D. Baptista-Hon: None. F. Robertson: None. T.G. Hales: None.

Poster 477. GABAA Receptors

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 477.19/A95

Topic: B.02. Ligand-Gated Ion Channels

Title: The effect of varying ratios of $\alpha_4$, $\beta_2$, and $\delta$ cRNA subunits on the receptor stoichiometry

Authors: *L. Y. HARTIADI, N. ABSALOM, P. K. AHRING, M. CHEBIB;
Fac. of Pharm., The Univ. of Sydney, Sydney, Australia

Abstract: $\delta$-containing GABAAR are exclusively expressed on extrasynaptic sites and mediate tonic inhibition. These receptors are important targets for neurosteroid, alcohol, and anesthetics and have been associated with numerous neurodegenerative and psychiatric disorders. Thus, $\delta$-containing GABAAR are potential therapeutic targets for drug development. However, significant variation in the pharmacology of these receptors is often reported in recombinant systems. We hypothesize that differences in pharmacology of $\delta$-containing receptors are due to variability in receptor stoichiometries. Two-electrode voltage clamp was used to study the influence of varying the amount of $\alpha_4$, $\beta_2$, and $\delta$ cRNA injected to Xenopus oocytes. Concentration-response curves (CRC) to GABA were measured along with the modulation and direct activation by $\delta$-selective compound 2 (DS2). GABA CRC at oocytes injected with $\alpha_4+\beta_2+\delta$ cRNA in ratios of 1:1:1, 1:1:5, 5:5:1, 1:5:1, and 1:5:5 had EC50 values ranging from 140 to 720 nM. 300 nM DS2 elicited large inward currents of 68-103% of the maximum GABA-elicited currents at these ratios. CRC of GABA at oocytes that were injected with 5:1:1 and 5:1:5
ratio of α4+β2+δ had higher EC50 values of 1.6 µM. The activation of DS2 on the 5:1:5 and 5:1:1 ratios were significantly lower than other ratios (P < 0.05, one-way ANOVA). At least two stoichiometries were expressed and each of the expressed stoichiometry responded differently to GABA and DS2. Under our conditions, varying the amount of α4 and β2 but not δ has a substantial role in the stoichiometry.


Poster

477. GABAA Receptors

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 477.20/A96

Topic: B.02. Ligand-Gated Ion Channels

Support: Swiss National Science Foundation grant 315230_156929/1

Title: Architecture and function of concatenated α4β2δ GABAA receptors

Authors: *N. WONGSAMITKUL, R. BAUR, E. SIGEL;
Univ. of Bern, Bern, Switzerland

Abstract: γ-aminobutyric acid (GABA)₅ receptors play an important role in inhibitory transmission in the mammalian brain. They are composed of five subunits surrounding a chloride channel. The major isoform of GABA₅ receptor are composed of two α, two β and one γ subunit(s). In many extrasynaptic receptors the γ subunit is replaced by δ. Diverse results were obtained with heterologously expressed α4β2δ receptors presumably because free α4, β2 and δ subunits can assemble into various subunit arrangements, depending on experimental conditions. We used concatenated receptors in which subunit configurations were predefined. α4, β2 and δ subunits were concatenated to dimeric and trimeric subunit constructs. Five pentameric GABA₅ receptors were built from the combination of trimers and dimers. The δ subunit was placed in any of the five positions in the α1β2γ2 receptor with γ2 replaced by β2. The resulting receptors were expressed in Xenopus oocytes and their electrophysiological properties were characterized using the two-electrode voltage clamp technique. Several combinations did not result in functional receptors. We chose only subunit combinations in which concatenated receptors elicited high current amplitudes to the agonist GABA in combination with the neurosteroid 3α, 21-dihydroxy-5α-pregn-20-one (THDOC). In control experiments, it was tested if trimers or dimers alone formed functional receptors. We also determined the effects of the δ-selective positive modulator 4-chloro-N-[2-(2-thienyl)imidazo[1,2-α]pyridine-3-yl benzamide (DS2) on
functional concatenated receptors. We furthermore tested ethanol effects on the functional concatenated receptors. By using receptor subunit concatenation, we could derive the putative architecture of α4β2δ GABA_{A} receptors.

**Disclosures:** N. Wongsamitkul: None. R. Baur: None. E. Sigel: None.

**Poster**

**477. GABAA Receptors**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 477.21/A97

**Topic:** B.02. Ligand-Gated Ion Channels

**Title:** Modulatory effects of neurosteroids and thyroid hormones on GABA-evoked currents in cultured dorsal root ganglion cells

**Authors:** *G. PUIA, L. RAVEGNANI, F. RAVAZZINI, R. AVALLONE, R. BARDONI; University of Modena and Reggio Emilia, Modena, Italy

**Abstract:** Neurosteroids (NSs) and Thyroid hormones (THs, T3, triiodothyronine and T4, thyroxine) are important endogenous modulators of GABAA receptor (GABAAR) function (Puia and Losi, 2011). The involvement of NSs in several physiological and pathological processes has been largely acknowledged, among them pain transmission. Several studies revealed the antinociceptive properties of some NSs and demonstrated that they can induce a potent peripheral analgesia via a direct GABAAR allosteric modulation (Poisbeau et al., 2014). Very little is known instead of how THs affect synaptic transmission in structures devoted to pain transmission. Pain sensitivity is related to the thyroid status, indeed hyperthyroidism confers greater sensitivity to thermal noxious stimuli (Edmondson et al., 1990) and alters the nociceptive responses in rats (Bruno et al., 2005). Dorsal root ganglion (DRG) cells are primary sensory neurons playing important roles in pain transmission between periphery and CNS. By using the patch clamp technique in the whole cell configuration we analyzed the effect of THs and of some NSs (Pregnenolone Sulfate, PS, and Allopregnenolone, ALLO) on GABA-evoked currents in rat DRG cells grown in primary cultures T3, T4 and PS (from 500 nM to 50 µM) reduce GABA-evoked currents with an IC50 of 0.8±0.3µM for T3 (eff max =-42±9%), of 1.4±0.7 µM for T4 (eff max =-41±6%) and of 4.3±1.2µM (eff max =-60±8%) for PS. ALLO potentiates GABA-evoked current in DRG neurons with an IC50 of 1.3±0.8 µM and a maximal effect of 110±20%. To investigate the mechanism of action of THs and PS we applied increasing concentrations of GABA (5, 10 and 50 µM) to the same concentration of modulator (10 µM). The effect of T3, T4 and PS was not dependent on the GABA concentration used suggesting that they act in a non-
competitive way. The modulatory activity of THs and PS on sIPSCs amplitude and frequency measured in lamina II neurons of acutely dissociated spinal cord slices was also investigated. In conclusion, since DRG neurosteroidogenesis is a physiologically relevant process (Schaeffer et al., 2010), our findings suggest that NSs modulation of GABAAR in this cells could play an important role in pain transmission from periphery to spinal cord. Furthermore the decreased GABAAR activity induced by T3 and T4 result in a reduced inhibitory neurotransmission that could contribute to the increased pain sensitivity detected in hyperthyroid animals.

Disclosures: G. Puia: None. L. Ravegnani: None. F. Ravazzini: None. R. Avallone: None. R. Bardoni: None.

Poster

477. GABAA Receptors

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 477.22/A98

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH grant R01 EY018441

Title: Regulation of Inhibitory neurotransmitter GABAA receptor subunit genes by nuclear respiratory factor 2 in neurons

Authors: *B. A. NAIR, M. WONG-RILEY;
Cell Biology, Neurobio. and Anat., Med. Col. of Wisconsin, Milwaukee, WI

Abstract: Nuclear respiratory factor 2 (NRF-2), or GA binding protein (GABP), is a unique transcription factor among E-26 transformation-specific (ETS) family, as the transcriptionally active complex is an obligate heterodimer or heterotetramer that is composed of two distinct proteins. The α subunit contains the ETS DNA binding domain that binds to the ‘GGAA’ or ‘TTCC’ motif, and the β subunit contains the transcriptional activation domain. Previous studies in our laboratory have revealed that NRF-2 tightly couples neuronal activity and energy metabolism by transcriptionally co-regulating all 13 subunits of an important energy-generating enzyme, cytochrome c oxidase (COX), as well as critical subunits of excitatory NMDA receptors such as Grin1 and Grin2b and Gria2 of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors. What is unknown is whether NRF-2 also regulates inhibitory neurotransmitter receptor genes to achieve a more balanced transmission in neurons. To test our hypothesis that NRF-2 also regulates GABA, specifically GABAA receptor subunit genes, we used multiple approaches, including in silico analysis, electrophoretic mobility shift and
supershift assay (EMSA), chromatin immunoprecipitation (ChIP), real-time quantitative PCR, and western blots. We found that NRF-2 functionally regulates the transcription of GABAA receptor subunit genes. Interactions of NRF-2 with GABAA subunit promoters were verified with in vitro EMSA and in vivo ChIP assays of mouse visual cortical chromatin. Transient transfections with NRF-2 over-expression or shRNA vectors in cultured neurons indicated that over-expression increased the mRNA and protein levels of GABAA subunit genes, whereas silencing reduced their levels, indicating a positive regulation by NRF-2. The binding sites of NRF-2 are conserved among rats, mice, and humans. Thus, our results indicate that NRF-2 plays a key role in regulating the transcription of GABAA receptor subunit genes, and that it aids in maintaining a transcriptional balance between excitatory and inhibitory synaptic transmission in neurons.

Disclosures: B.A. Nair: None. M. Wong-Riley: None.

Poster

477. GABAA Receptors

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 477.23/A99

Topic: B.02. Ligand-Gated Ion Channels

Title: New tools to characterize allosteric modulators at the GABAA receptors

Authors: *S. BERTRAND, E. NEVEU, D. BERTRAND; Hiqscreen, Vesenaz - GE, Switzerland

Abstract: Studies of the ionotropic ligand gated ion channels activated by the γ-aminobutyric acid (GABA) require the possibility to effectuate many experimental protocols ranging from the simple concentration-activation curves to more elaborate paradigms to evaluate the potency and efficacy of allosteric modulators. In this work we present new tools for testing ligand gated ion channels and allosteric modulators using a fully automated recording system that performs unattended experiments. GABAA receptors expressed in Xenopus oocytes using recombinant human cDNA’s or cRNA’s were automatically injected in oocytes using a micro syringe driven system (roboinject from Multichannel Systems). Recordings were conducted using the automated two electrodes recording system HiClamp (Multichannel Systems) and proprietary software (HiQScreen Sàrl). Determination of the concentration activation curves for different GABAA receptor subtypes including the α1β2γ2, α5β3γ2 or α4β3δ confirmed the adequation of protein expression as well as quality of the compound application. Specific experimental protocols were then designed to assess the effects of allosteric modulators of the GABAA
receptors including benzodiazepine, neurosteroids or zinc. The availability of a fully automated system allowed testing of a single concentration of a compound and comparison with control response on a single cell. These procedures were successfully employed to characterize the concentration-response of a series of allosteric modulators at different GABAA receptors and to establish the “fingerprint” of each receptor for a given sets of compounds. The good correlation between data obtained using this system versus results presented in the literature confirmed the value of these tools to further evaluate ligand gated ion channels. Moreover, this method allows a direct comparison of a series of allosteric modulator at different human GABAA receptors revealing the uniqueness of compounds that is of value for the development of new therapeutic treatments.


**Poster**

**477. GABAA Receptors**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 477.24/A100

**Topic:** B.02. Ligand-Gated Ion Channels

**Title:** Zolpidem enhances GABA-induced currents at (α1)3(β3)2 GABAA receptors via a novel binding interface

**Authors:** *A. T. CHE HAS*¹, N. ABSALOM¹, A. CLARKSON², P. AHRING¹, M. CHEBIB¹; ¹Fac. of Pharm., Univ. of Sydney, Camperdown, Australia; ²Dept. of Anat., Univ. of Otago, Otago, New Zealand

**Abstract:** Zolpidem, an imidazopyridine, is one of the most widely used medications for sleep. Acting at GABAA receptors, it is known to bind between an α1+/γ2- subunit interface with preference for α1 subunit-containing GABAA receptors. Previous studies have found that the α1+/β3- interface is also capable of binding certain benzodiazepine site ligands. Therefore, we investigated the possibility of whether binary GABAA receptors composed of α1β3 subunits exist in different stoichiometries and whether zolpidem can modulate one stoichiometry over another. Two-electrode voltage clamp was used to study the effects of GABA and zolpidem at binary α1β3 GABAA receptors expressed in Xenopus laevis oocytes formed after injecting different α1:β3 cRNAs ratios (30:1 or 1:1). To ensure receptors contain an α1+/α1- interface, we also injected β3-α1 concatamers together with individual α1 subunit cRNAs. Currents from all
expressed receptors were recorded with either GABA or GABA EC5 co-applied with zolpidem. Different GABA A receptor stoichiometries composed of α1β3 were formed when we varied the cRNA injection ratio of α1:β3. Receptors that formed after injecting a 30:1 cRNA ratio were less sensitive to GABA (EC50 31 µM). In the presence of GABA EC5, the maximal concentration of zolpidem (1 µM) enhanced the GABA-induced current by 116%. The enhancement was blocked by 1 µM flumazenil. In contrast, α1β3 receptors formed from the 1:1 injection ratio were more sensitive to GABA (EC50 2.7 µM) and these receptors were insensitive to zolpidem enhancement. GABA A α1β3 receptors formed from injecting cRNA of β3-α1 concatamers with the α1 subunit closely matched the characteristics of receptors expressed from the 30:1 injection ratio. Zolpidem similarly enhanced the GABA-induced current, demonstrating that α1β3 receptors that contain an α1+/α1- interface are positively modulated by zolpidem. The α1+/α1- interface is a unique site and may mediate in part the physiological effects of zolpidem.


Poster

477. GABA A Receptors

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 477.25/A101

Topic: B.02. Ligand-Gated Ion Channels

Title: Characterisation of the pharmacological actions of kavain at GABA A Rs

Authors: *H. C. CHUA 1, K. HOSTGAARD-JENSEN 2, E. CHRISTENSEN 1,2, A. JENSEN 2, I. RAMZAN 1, N. ABSALOM 1, M. CHEBIB 1;

1Fac. of Pharm., University of Sydney, Sydney, Australia; 2Dept. of Drug Design and Pharmacol., Univ. of Copenhagen, Copenhagen, Denmark

Abstract: Kavain is one of the major kavalactones found in kava, a beverage prepared from the pepper plant Piper methysticum commonly consumed among the Pacific islanders and indigenous Australians for its anxiolytic and sedative properties. The mechanism of action of kavalactones remains elusive, but γ-aminobutyric acid type A receptors (GABA A Rs) have been proposed to be a possible target. In this study, we evaluated the action of kavain at various GABA A R subtypes expressed in Xenopus oocytes using the two-electrode voltage clamp technique. We found that kavain positively modulated α1β2γ2L receptors, but only at GABA concentrations below the EC45. This enhancement effect does not require the α1 or the γ2L subunit as kavain also positively modulated α1β2 and β2γ2L binary receptors to similar extent.
Kavain showed poor selectivity as it was found to have comparable action across αxβγ2L (where x = 1, 2, 3 and 5) and α1β1γ2L receptors. In an attempt to identify the potential binding site(s) of kavain, we co-applied various GABA_A,R ligands with kavain at α1β2γ2L receptors. Flumazenil (10 μM) failed to antagonise the enhancement effect of kavain, further supporting the findings of previous studies that kavalactones act via a non-benzodiazepine mechanism. The co-application of GABA, kavain and other positive modulators such as diazepam, etomidate, allopregnanolone and propofol resulted in less-than-additive enhancement, suggesting the modulatory pathway of kavain might overlap with other allosteric ligands. In summary, this study demonstrates that kavain directly modulates GABA_A,Rs via a novel, unknown mechanism. As kavalactones have been shown to have antianxiety effect with no addictive or withdrawal issues in clinical trials, understanding their pharmacology at GABA_A,Rs may aid in the development of more effective therapeutics.


Poster

477. GABAA Receptors

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 477.26/A102

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH Grant R01AA010983

NIH Grant U01AA019967

NIH Grant F31AA002843

NIH Grant K01AA022475

Title: Adolescent alcohol exposure induces layer-specific deficits in δ-GABAA receptor-mediated tonic currents in the adult prelimbic cortex

Authors: *S. CENTANNI, E. J. BURNETT, H. TRANTHAM-DAVIDSON, L. J. CHANDLER;
Neurosci., Med. Univ. of South Carolina, Charleston, SC

Abstract: The goal of the current study was to investigate the effect of adolescent intermittent ethanol (AIE) exposure on the adult GABAergic system in the prelimbic cortex (PrL-C). Long-
Evans rats were subjected to 4 cycles of AIE exposure by vapor inhalation between PD28-42. In control rats, patch-clamp electrophysiology in acute slices obtained at different postnatal ages revealed a developmental increase in the GABAA receptor-mediated tonic current in layer V pyramidal neurons. In slices from AIE-exposed rats, the amplitude of the tonic current was significantly reduced compared to controls when tested at PD 45, 60 and 90-120. This AIE-induced reduction in tonic current was found to reflect attenuation of currents mediated by δ-subunit containing receptors using the δ-GABAA receptor agonist THIP (1µM). This reduction in tonic current in the adult was observed in both male and female AIE-exposed rats. Interestingly, AIE did not alter tonic or THIP-mediated currents in layer II/III PrL-C, indicating the effect of AIE is specific to layer V pyramidal neurons in the PrL-C. In addition, facilitation of the tonic current by bath application of either ethanol or allopregnanolone was attenuated in slices from AIE-exposed adult rats compared to control rats. Immunohistochemistry and western blot analysis revealed no change in the expression of δ-GABAA subunits or their surface expression suggesting that the AIE-induced reduction in tonic current may relate to posttranslational modification that reduce channel conductance. Lastly, the critical role GABA plays in neural network synchronization and PFC-dependent cognitive function led to the hypothesis that AIE produces deficits on a probabilistic decision-making task in adulthood. Surprisingly, although AIE exposure resulted in deficits in some measures of behavioral flexibility associated with prefrontal function, we did not observe changes in performance on a probabilistic decision-making task. Taken together, these studies reveal that AIE exposure results in persistent deficits in δ-GABAA receptor-mediated tonic currents in the adult PrL-C that may contribute to deficits in PFC-dependent behavioral control in adulthood.


Poster

477. GABAA Receptors

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 477.27/A103

Topic: B.02. Ligand-Gated Ion Channels

Support: Swiss National Science Foundation grant 315230_156929/1

M.C.M. is a recipient of a fellowship (Beca Chile Postdoctorado from CONICYT, Ministerio de Educacion, Chile)

Title: Novel compounds with anesthetic activity
**Authors:** R. BAUR\(^1\), M. C. MALDIFASSI\(^1\), S. A. FORMAN\(^2\), *E. SIGEL\(^1\);
\(^1\)Univ. Bern, CH-3012 Bern, Switzerland; \(^2\)Anesthesia Critical Care & Pain Med., Massachusetts Gen. Hosp., Boston, MA

**Abstract:** Recently, we described experiment-guided virtual screening (EGVS), a method that was used to identify new ligands of the classical benzodiazepine binding pocket located at the extracellular α+/γ- subunit interface in α₁β₂γ₂ GABA\(_A\) receptors (Middendorp et al., ACS Chem. Biol., 2014). Diazepam interacts with high affinity with this site 1 and low affinity with the ill defined site 3 in the membrane (Walters et al., Nature, 2000). Site 3 probably corresponds to the large cavity homologous to the cavity occupied by ivermectin in *C. elegans* GluCl receptors. GluCl S260, homologous to the residue β₂265, forms an H-bond with ivermectin (Hibbs and Gouaux, 2011). Site 3 has earlier been shown to be abolished upon combined mutation of residue β₂265 and homologous residues in other subunits (α₁S269I, β₂N265I, γ₂S280I). The pharmacophore describing site 1 would be expected to also relate to site 3. Indeed, a number of compounds acting at this low affinity site were identified. First we determined the concentration response curves for all of the new compounds at α₁β₂γ₂ GABA\(_A\) receptors expressed in Xenopus oocytes. Subsequently we studied the effect on modulation of individual sites 3 mutations (α₁S269I, β₂N265I and γ₂S280I). While all of the new compounds were sensitive to the mutation in β₂, they differed in their reaction on the mutations in the α₁ and γ₂ subunit. New compounds were compared with propofol and etomidate. While modulation of propofol and etomidate were abolished by the mutation in the β₂ subunit, the mutation in the α₁ subunit did not change the response. Moreover the γ₂ mutation had different consequences for each, potentiation by propofol was reduced, whereas that by etomidate was increased. New compounds were tested *in vivo* for their anesthetic activity. LORR (loss of righting reflex) was determined in tadpoles at a concentration of 10 µM. In addition, a number of published modulators were assessed. Several of the compounds displayed anesthetic activity. In summary, we describe here novel potential anesthetics.

**Disclosures:** R. Baur: None. M.C. Maldifassi: None. S.A. Forman: None. E. Sigel: None.

**Poster**

477. GABA\(_A\) Receptors

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 477.28/A104

**Topic:** B.02. Ligand-Gated Ion Channels

**Support:** AA017072
Title: Postnatal development of subcompartmental inhibition in prefrontal layer 2/3 pyramidal cells

Authors: G. RINETTI VARGAS¹, *K. J. BENDER²;
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Abstract: Behaviors that engage prefrontal cortical (PFC) circuits continue to mature through adolescence. This development parallels PFC network maturation, including the continued refinement of local GABAergic interneuron signaling. Chandelier cells are a subclass of parvalbumin positive interneuron that synapses exclusively on the axon initial segment of local excitatory pyramidal cells. These axo-axonic cells are thought to regulate large, high-order PFC networks, as their dendrites sample associative inputs from other cortical regions, and their axons diverge to synapse on hundreds of pyramidal cells. Chandelier cell maturation is thought to be critical for proper PFC development, as they undergo extensive anatomical refinement during adolescence. Whether they also undergo similar functional maturation during adolescence is unclear. While somatodendritic GABAergic synapses develop to hyperpolarize local membrane voltage after the first postnatal week, some reports indicate that prefrontal axo-axonic synapses continue to depolarize postsynaptic pyramidal cells well past this time point. Whether this suggests that chandelier cells act to excite pyramidal cell networks in mature PFC, or that these synapses mature to a more traditional inhibitory role far later than neighboring somatodendritic synapses is unclear. Here, we assayed the postsynaptic actions of dendritic and AIS-targeting inhibitory synapses throughout postnatal PFC development using 2-photon guided GABA iontophoresis targeted to pyramidal cell neurites. We found that chloride reversal potentials (E_Cl) could vary markedly between the axon and dendrite of layer 2/3 pyramidal cells in brain slices from preadolescent mice. Dendritic GABA_A receptor activation consistently shunted or hyperpolarized the membrane throughout adolescent development. In contrast, GABAergic potentials in the axon initial segment underwent a significant shift during adolescence, with depolarizing responses from resting Vm in neurons from pre-adolescent mice, and shunting or hyperpolarizing responses in neurons from adult mice. We are currently exploring the cellular mechanisms that underlie this adolescent shift in GABAergic function in the axon. These data define an adolescent period for axo-axonic GABAergic synapse development that is significantly delayed compared to neighboring synapses onto dendrites, and may define a major cellular component of PFC maturation during this time period.

Disclosures: G. Rinetti Vargas: None. K.J. Bender: None.

Poster

478. Ion Channels

Location: Hall A
Title: NR1/NR2A and NR1/NR2B NMDA receptor profiling in high-throughput patch clamp

Authors: G. KIRSCH, N. FEDOROV, Y. KURYSHEV, L. ARMSTRONG, *C. MATHES, A. M. BROWN; Chantest Corp., Cleveland, OH

Abstract: Ionotropic glutamate NMDA receptors play fundamental roles in synaptic plasticity, memory and learning, and increasingly are targeted in drug discovery for neuropathic pain, ischemic stroke, Parkinson’s disease, Alzheimer disease, and several forms of dementia. Multiple receptor isoforms with distinct brain distributions and functional properties arise by selective NR1 transcript splicing and differential expression of NR2(A-D) subunits. NR1/NR2A and NR1/NR2B represent the most abundant subtypes with well-documented biophysical properties and pharmacology in conventional, low throughput voltage clamp electrophysiology. Exploitation of recent advances in high-throughput patch clamp technology in drug discovery, however, requires assay optimization and characterization of the receptor subtypes. In this study we used IonWorks Barracuda, a high-throughput instrument that operates in 384-well format, population patch clamp (PPC) mode. Procedures were optimized to routinely obtain signals 1 - 2 nA (PPC mode, Z’ values >0.6) in human NR1/NR2A and NR1/NR2B receptors stably expressed in HEK293 cells. Side-by-side comparisons of the pharmacological properties of human NR1/NR2A and NR1/NR2B receptors were performed with agonists, positive modulators, and competitive or uncompetitive inhibitors. We found that EC50 values for L-glutamate were 3.8 µM and 2.0 µM for NR1/NR2A and NR1/NR2B, respectively. NMDA behaved as a partial agonist at NR1A/NR2B (Emax ≈ 75% and EC50=18-20 µM), whereas NMDA was a full agonist at NR1/NR2A (Emax ≈ 100%, EC50 = 100 µM). The EC50 values were consistent with literature data obtained with conventional manual patch clamp methods. As expected, glycine and D-cycloserine produced concentration-dependent potentiation of glutamate activation. Additionally, we confirmed subtype selectivity of the uncompetitive inhibitor, ifenprodil. NR1/NR2B receptors were inhibited in a concentration-dependent manner with IC50 = 19.3 µM, whereas NR1/NR2A receptors were unaffected. Our results demonstrate the potential of robust, high-throughput patch clamp assays for profiling new chemical entities against NMDA receptor subtypes.

Disclosures: G. Kirsch: A. Employment/Salary (full or part-time); ChanTest Corp. N. Fedorov: A. Employment/Salary (full or part-time); ChanTest Corp. Y. Kuryshev: A. Employment/Salary (full or part-time); ChanTest Corp. L. Armstrong: A. Employment/Salary (full or part-time); ChanTest Corp. C. Mathes: A. Employment/Salary (full or part-time); ChanTest Corp. A.M. Brown: A. Employment/Salary (full or part-time); ChanTest Corp.
Title: Involvement of ENaC on initiation of mechanically-evoked swallows in anesthetized rats

Authors: *T. TSUJIMURA, K. TSUJI, S. SAKAI, T. SUZUKI, M. INOUE; Niigata Univ. Grad. Sch. of Med. and Dent. Sci., Niigata City, Japan

Abstract: Purpose: Mechanical stimulation to pharyngolaryngeal regions can readily evoke swallowing reflex which plays a role as airway defensive reflex. Because disordered laryngopharyngeal sensation is significantly related to the development of aspiration pneumonia, it is important to know how laryngeal sensory system regulates swallowing. The aim of this study was to investigate the role of amiloride-sensitive epithelial sodium channel (ENaC) in mechanically evoked swallows. Materials and Methods: Male Sprague Dawley rats anesthetized with urethane were used. Swallows were identified by supra- and infrahyoid electromyographic bursts accompanied by transient decreases in upper airway (UA)-flow or increases in esophageal pressure. Amiloride analogues (amiloride, benzamil, dimethylamirolide), ASIC inhibitors (mambalgine-1, diminazene) and gadolinium were applied topically (0.3-30 nmol, 3µl) to vocal folds. Swallowing threshold was measured for von Frey filament or electrical stimulation to vocal folds (n = 4-6 in each group). The number of swallows by capsaicin application (0.03 nmol, 3µl) to vocal folds or UA distension were also measured (n = 5 and 6, respectively). Results & Discussion: The mechanical swallowing threshold to larynx was significantly increased by glossopharyngeal (IX) and superior laryngeal (SLN) nerves transection (Control; median 0.008 [IQR, 0.008], IXx and SLNx; median 0.02 [IQR, 0.02-0.4], n=5 in each group) and the swallowing was completely abolished by IX, SLN, recurrent laryngeal and pharyngeal branch of vagus nerves transection. The mechanical threshold of swallowing was increased in a dose-dependent manner of amiloride analogues and gadolinium, but not ASIC inhibitors. The increased swallowing threshold by amiloride analogues and gadolinium was recovered after saline (10 µl) washout. The number of swallows by UA distention was significantly decreased following benzamil application (Control; 8.2±0.7 vs. 30 nmol Benzamil; 1.0±0.5). Benzamil unchanged swallow-related muscle activities in UA-evoked swallows. On the other hand, benzamil did not affect the initiation of swallows evoked by capsaicin and electrical stimulation.
Conclusion: We speculate that ENaC is involved in initiation of mechanically evoked swallows in larynx.


Poster

478. Ion Channels

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#/Poster#: 478.03/A107

Topic: B.04. Ion Channels

Support: DFG GRK 1482

Title: Osmosensitivity in the enteric nervous system

Authors: *P. KOLLMANN, M. SCHEMANN, G. MAZZUOLI-WEBER;
Human Biol., Technische Univ. München, Freising, Germany

Abstract: Background/Objective: the enteric nervous system (ENS) is made up of hundreds million neurons having a distinctive property compared to all other neurons. They are located directly inside the wall of the gastrointestinal tract, in a key position, that allows them to sense and control all the gut physiological functions as secretion and motility. After water or food intake the osmolarity in the gastrointestinal lumen as well as in the blood undergoes profound changes. Earlier studies already revealed that luminal shift of osmolality alter gastrointestinal motility. Our hypothesis is that enteric neurons and/or glia which are strategically located between blood vessels and epithelial cells may sense and respond to intramural osmolarity changes. Methods: we used fast neuroimaging techniques with calcium or voltage sensitive dyes to record immediate reactions from neurons of the submucous and myenteric plexus ex vivo, in intact tissue preparations, and in vitro from primary cultured myenteric neurons. As a stimulus we briefly exposed the tissues/cells to hyper- or hypoosmolar stimuli (200 mOsm/Kg - 400 mOsm/Kg) by a controlled local perfusion system. With this method we were able to detect either changes in intracellular [Ca2+] or action potentials fired by the neurons. Volume changes of the neurons were also calculated. Immunohistochemistry was applied to characterize the responding cells. Results: here we report preliminary data on the response of enteric neurons to hypoosmolar as well as hyperosmolar stimulation. Cultured myenteric neurons showed an increase in intracellular [Ca2+] after local perfusion with hypoosmolar solution. They also showed an immediate and transient increase in volume (10% area increase, 236 mOsm/Kg)
which peaked simultaneously with the peak in intracellular [Ca2+]. In the intact tissue preparations 14.4% of the neurons of the submucous plexus fired action potentials with a mean frequency of 1.64 Hz after a brief hypoosmolar (-100 mOsm/Kg) stimulation. A similar proportion of submucous enteric neurons (15.0%) showed responses to hyperosmolar stimuli (+100 mOsm/Kg). These neurons fired with a mean frequency of 0.81 Hz after the stimulus application. Conclusion: The ENS contains cells that respond to hypoosmolar as well as hyperosmolar solutions with increased intracellular [Ca2+] and action potential discharge. These findings reveal for the first time that the enteric neurons are capable of sensing and react to changes in intramural osmolarity. Further experiments are needed to provide data for the functional relevance of this new enteric neuronal property.


Poster

478. Ion Channels

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 478.04/A108

Topic: B.04. Ion Channels

Support: TUBITAK Grant: SBAG-110S397

Title: TRPM2 channels in octopus neurons of mice cochlear nucleus

Authors: *R. BAL*, E. O. ETEM, Y. MORI;
1Gaziantep Univ., Gaziantep, Turkey; 2Med. Biol., Firat Univ., Elazig, Turkey; 3Dept. of Synthetic Chem. and Biol. Chem., Kyoto Univ., Kyoto, Japan

Abstract: TRPM2 is a Ca2+-permeable cation ion channels, which is member of the transient receptor potential melastatin family. TRPM2 is activated by reactive oxygen/nitrogen species (ROS/RNS) and ADP-ribose (ADPR) and is linked to cell death. In the present study, we aimed to study if TRPM2 channels are present in the octopus cells of mice posterioventral cochlear nucleus, if present, to characterize the biophysical and pharmacological properties and function of TRPM2 channels in octopus neurons. mRNA expression for TRPM2 were demonstrated using quantitative real-time reverse transcription-polymerase chain reaction (qRT-PCR), TRPM2 ion channel proteins were demonstrated by western blotting and also spatial localization of TRPM2 ion channel proteins on the cellular membrane of octopus neurons were visualized by immunohistochemical staining technique. Whole-cell patch clamp recordings were performed under current and voltage clamp condition in coronal slices from the mice cochlear nucleus
TRPM2 agonists including ADP-ribose (ADPR) induced a small degree of depolarization in octopus cells, which were blocked by TRPM2 antagonists, flufenamic acid (100 μM), N-(p-amylcinnamoyl) anthranilic acid (50 μM) and 8-Bromo-cADP ribose (50 μM). In the octopus cells of the TRPM2- knock out mice, application of ADPR in the pipet caused significantly less depolarization of the resting membrane potential, 0.45±0.21 mV, which was not affected by specific TRPM2 antagonists (n=5). It is concluded that TRPM2 channels seems to be present and function to set the membrane potential in a depolarization direction in octopus cells of cochlear nucleus. This work was supported by a grant from TUBITAK (SBAG-110S397).

Disclosures: R. Bai: None. E.O. Etem: None. Y. Mori: None.
VSP). The inhibition of KCNQ current by low concentrations of M1R agonists was delayed in cells treated with myo-inositol and SMIT1. Comparable results were observed for GIRK2 and TRPM7 channels, suggesting a mechanism of increased intracellular PIP2 levels. Myo-inositol supplementation also speeded the return of fluorescent probes for PI(4,5)P2 to the plasma membrane following brief PI(4,5)P2 depletion. Further, other experiments using the rapamycin-recruitable phosphatase Sac1 and the P4M probe to specifically hydrolyze or visualize PI(4)P suggested that PI(4)P levels increased after myo-inositol supplement with SMIT1. Elevations of PIP and PIP2 were directly confirmed by using a triple quadrupole mass spectrometer to assess relative levels of phosphoinositides. As might be expected, the IP3 metabolism and IP3-mediated intracellular calcium release were also altered after myo-inositol supplement, consistent with the notion of elevated Pl(4,5)P2 levels. Finally, we found that 24-h treatment with hypertonic solutions imitated the phenomena we observed with SMIT1 overexpression whereas pharmacological or siRNA treatment against tonicity-responsive enhancer binding protein (TonEBP) partially reversed the effects. These results show that ion channel function and cellular excitability are under regulation by several "physiological" manipulations that alter the Pl(4,5)P2 setpoint of cells.


Poster

478. Ion Channels

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 478.06/B2

Topic: B.04. Ion Channels

Title: Electrophysiological properties of mechanosensitive neurons of rat dorsal root ganglia

Authors: *W. CHANG1, V. VIATCHENKO-KARPINSKI2, H. KANDA2, J. LING2, J. GU2; 1Univ. of Alabama At Birmingham, Birmingham, AL; 2Anesthesiol., Univ. of Alabama at Birmingham, Birmingham, AL

Abstract: Sensing mechanical stimuli is important in life but mechanisms underlying the transduction and processing of mechanical stimuli in the somatosensory system remain poorly understood. Mechanically activated (MA) ion channels and currents have been identified in somatosensory neurons including dorsal root ganglion (DRG) neurons and trigeminal ganglion neurons. DRG neurons that express MA channels not only play a role in mechanical transduction under physiological conditions, but also may be involved in chronical mechanical pain under
pathological conditions. In the present study, we used acutely dissociated DRG neurons and applied whole-cell patch-clamp recording technique to study MA currents and electrophysiological properties of DRG neurons that show MA currents. Cells were tested under both current-clamp and voltage clamp modes and classified by their sensitivity to mechanical stimulation, types of mechanically activated currents, profiles of voltage-activate currents, action potential firing properties and other electrophysiological properties. Obtained results may help us to further understand how mechanical stimuli are processed by the somatosensory system.

Disclosures: W. Chang: None. V. Viatchenko-Karpinski: None. H. Kanda: None. J. Ling: None. J. Gu: None.

Poster

478. Ion Channels

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 478.07/B3

Topic: B.04. Ion Channels

Support: Grant-in-Aid for Scientific Research, KAKENHI 24240045

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Strategic Research Foundation Grant-aided Project for Private Universities from Ministry of Education, Culture, Sport, Science, and Technology, Japan (MEXT), 2014-2018 (S1411003).

Title: Magnesium influx triggered by neural depolarization

Authors: *R. YAMANAKA, Y. SHINDO, T. KARUBE, R. TANAMOTO, K. HOTTA, K. SUZUKI, K. OKA;
KEIO UNIVERSITY, Yokohama, Kanagawa, Japan

Abstract: Magnesium ion (Mg²⁺) is involved in a wide variety of biochemical reactions, and a multitude of physiological functions are known to require Mg²⁺. Many reports suggest that Mg²⁺ deficiency is also involved in various neuronal diseases. Therefore, Mg²⁺ dynamics in neurons may play key roles in healthy neuronal functions. In facts, main excitatory neurotransmitter glutamate induced intracellular Mg²⁺ concentration ([Mg²⁺]i) increase in neurons. However, it has not been revealed whether neuronal activity under physiological condition modulate [Mg²⁺]. This study aimed to explore the direct relationship between neural activity and [Mg²⁺]i dynamics. In rat primary dissociated hippocampal neurons, the [Mg²⁺]i and [Ca²⁺]i dynamics were simultaneously visualized by a highly selective fluorescent Mg²⁺ probe KMG-104 and a
fluorescent Ca\textsuperscript{2+} probe Fura Red, respectively. The [Mg\textsuperscript{2+}], increase was observed at the timings of spontaneous Ca\textsuperscript{2+} increase in neurons whose excitability was enhanced by treatment of voltage-gated K\textsuperscript{+} channel blocker, TEA. And, the [Mg\textsuperscript{2+}] increase was abolished in Mg\textsuperscript{2+}-free extracellular medium, indicating [Mg\textsuperscript{2+}] increase is due to neural activity-induced Mg\textsuperscript{2+} influx. Next, to verify the occurrence of [Mg\textsuperscript{2+}] increase concomitant with neural activity by non-pharmaceutical paradigm, we plated neurons on glasses coated with electrically conductive and optically transparent indium-tin oxide (ITO) films, which enables fluorescent imaging during neural stimulation. The [Mg\textsuperscript{2+}] increase was detected at the timing of Ca\textsuperscript{2+} response with corresponding stimulation. The direct neuronal depolarization by veratridine, Na\textsuperscript{+} channel opener, application induced [Mg\textsuperscript{2+}] increase, and the [Mg\textsuperscript{2+}] increase was suppressed by the pretreatment of non-specific Mg\textsuperscript{2+} channel opener, 2-APB. Overall, neural depolarization induced [Mg\textsuperscript{2+}] influx from extracellular via 2-APB sensitive channel in rat hippocampal neurons.


Poster

478. Ion Channels

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 478.08/B4

Topic: B.04. Ion Channels

Support: Canadian Institutes of Health Research (CIHR Grant # 130530)

Canadian Institutes of Health Research (CIHR Grant # 125901)

Research Manitoba Postdoctoral Fellowship

Title: A PANX1 loss-of-function mutation identified from a patient with intellectual disability, hearing loss and endocrine disorders

Authors: *R. SHI\textsuperscript{1,2}, Q. SHAO\textsuperscript{3}, K. LINDSTROM\textsuperscript{4}, J. KELLY\textsuperscript{3}, A. SCHROEDER\textsuperscript{5}, J. JUUSOLA\textsuperscript{6}, K. LEVINE\textsuperscript{6}, J. L. ESSELTINE\textsuperscript{7}, S. PENUELA\textsuperscript{3}, M. F. JACKSON\textsuperscript{1,2}, D. W. LAIRD\textsuperscript{3};

\textsuperscript{1}Kleysen Inst. for Advanced Med., Winnipeg, MB, Canada; \textsuperscript{2}Dept. Pharmacol. & Therapeut., Univ. of Manitoba, Winnipeg, MB, Canada; \textsuperscript{3}Dept. Anat. & Cell Biol., Univ. of Western Ontario, London, ON, Canada; \textsuperscript{4}Div. of Genet. and Metabolism, Phoenix Children’s Hosp.,
Abstract: Aberrant activation of pannexin channels is associated with CNS disorders including ischemic injury, epileptiform bursting and cortical spreading depression underlying migraine aura. We now report the identification of a disease-linked PANX1 mutation in a 17-year-old female patient with intellectual disability, sensorineural hearing loss and other systemic dysfunctions, including skeletal defects (kyphoscoliosis) and primary ovarian failure. Whole exome sequencing identified a homozygous PANX1 gene mutation (c.650G>A) resulting in an arginine to histidine substitution at position 217 (R217H). Using a multidisciplinary approach the consequence of the R217H mutation on the expression and function of Panx1 channels was assessed. When expressed in N2A, NRK, HEK293T and Ad293 cells, the glycosylation and trafficking to the cell surface of R217H was unaltered when compared to wild type Panx1, suggesting that the surface expression of R217H is not impacted. To assess the functional consequence of the R217H mutation, we performed tight-seal whole-cell recordings from HEK293T cells expressing R217H or Panx1. In cells voltage-clamped to -60 mV, pannexin current amplitude and reversal potential was assessed from applied voltage-ramps (±100 mV, 500 ms). Current amplitude at +100 mV in cells expressing R217H was reduced by 50% compared to wild type Panx1. In contrast no change in reversal potential was noted suggesting that reduced currents in R217H expressing cells could not be attributed to a change in ionic permeability. Both R217H- and Panx1-mediated currents were augmented by high extracellular potassium treatment (50 mM KGluc) in cells expressing R217H, and Panx1-expressing cells were suppressed by the pannexin blocker carbenoxolone. As Panx1 is permeable to solutes of up to 1 kDa, the functional consequence of the R217H mutation on dye uptake and ATP release was determined. Uptake of ethidium bromide and release of ATP in response to high potassium treatment was impaired in cells expressing R217H. Collectively, our functional studies monitoring ion flux, dye uptake, and ATP release demonstrate that R217H, associated with developmental abnormalities in a human patient, represents a loss-of-function mutation. The first three authors contributed equally to this work.


Poster

478. Ion Channels

Location: Hall A
Title: Modulation of acid sensing ion channels by KB-R7943, a reverse Na+/Ca^{2+} exchanger inhibitor

Abstract: KBR-7943, an isothiourea derivative, is widely used as a pharmacological inhibitor of reverse sodium-calcium exchange (NCX). It has been shown to have neuroprotective effects in animal models of brain ischemia; however, the detailed mechanism remains elusive. In the current study, we investigated the effect of KB-R7943 on acid sensing ion channels (ASICs), a novel family of proton-gated cation channels involved in ischemic brain injury, with whole cell patch clamp techniques. We found that KBR-7943 potently inhibits ASIC currents in primary cultured mouse cortical neurons in a frequency-dependent manner with an effective concentration starting from 3 µM. KBR-7943 shows a similar frequency-dependent inhibition on ASIC1a current expressed in Chinese Hamster Ovary (CHO) cells. In contrast to the ASIC1a current, ASIC2a current was not inhibited by KBR-7943 with concentrations as high as 100 µM. Interestingly, KBR-7943 slightly inhibits the peak amplitude but slows down the desensitization of the ASIC3 current. Together, our data demonstrate that KBR-7943 modulates acid sensing ion channels in a subunit dependent manner. As inhibition of ASIC1a is known to be neuroprotective, our data suggest that the modulatory effects of KBR-7943 on ASIC1a might be an underlying mechanism for its neuroprotective effects against ischemic brain injury.

Disclosures: T. Leng: None. H. Si: None. Z. Xiong: None.
Title: On estimating ion channel densities in model neurons from simulated patch clamp data

Authors: *S. G. CARVER;
Mathematics and Statistics, American Univ., Washington, DC

Abstract: The need to estimate parameters creates a difficult challenge in studies of detailed biophysical models of neurons. Some parameters, such as those concerning the kinetics of single ion channels, can be measured (or fit with sufficient precision) from isolated channels. Other parameters are not so easy to determine. Specifically, the densities of the various ion channels remain, to date, difficult to infer in a principled way from biological data. A realistic model cell can have many dozens, even hundreds, of these density parameters - the values can vary from location to location across the cell membrane. This poster will analyze the statistical problem of fitting ion channel density parameters based on simulated patch clamp data. With simulated data, we already have the correct answer, and we can explore assumptions making the inference sufficiently tractable and successful to a given confidence level. By changing assumptions, we explore the boundary between hard problems and easy problems. We quantify “hard” by the amount of data needed to estimate the channel densities with confidence. Using Monte Carlo integration, we compute the probability of falsifying an alternative model (with different channel densities) in favor of the true model. The falsification succeeds if the true model's likelihood is greater than the alternative's. We ask how much data (i.e. how many repetitions of the protocol) are needed to correctly select the true model with a specified confidence level (e.g. 95%). Our model patch contains channels of one or more known types. Here "known" means that all alternative models use channels with the correct kinetics, without fitting parameters. From the patch clamp data, we infer only the numbers of ion channels present (in the patch) of each type. Each estimate of channel density is the corresponding estimated number of channels divided by the patch area.

Disclosures: S.G. Carver: None.

Poster

478. Ion Channels

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 478.11/B7

Topic: B.04. Ion Channels

Support: IWT-O&O-110448
Title: A novel selective α7 nicotinic acetylcholine receptor allosteric modulator showing concentration dependent positive and negative allosteric modulation

Authors: *K. VEYS;
Neurosci. R&D, Janssen Pharmaceutica, Beerse, Belgium

Abstract: We have characterized a novel compound showing non-competitive allosteric modulation of the α7 nicotinic acetylcholine receptor. Functionality was tested by whole cell patch clamp using a fast perfusion system on human α7 nicotinic acetylcholine receptors co-expressing ric-3 in a GH4C1 cell line. At low concentrations (0.03 mM) positive allosteric modulation was observed up to 140% on a near maximal acetylcholine response (1.3 mM) and up to 350% on a small acetylcholine response (0.1 mM). We did not observe any direct agonist activity of the compound with either electrophysiological or radioligand binding studies. A compound, EVP-6124, has been previously shown to have comparative modulating effects. Interestingly, in our own recordings, EVP-6124 showed no positive modulation nor did it show the alleged partial agonist activity. These discrepancies are believed to arise from the experimental approach that was used. Unfortunately, limited solubility caused difficulties in determining the crystal structure of the compound-receptor complex and therefore prevented further study of the compounds actual binding site(s).

Disclosures: K. Veys: A. Employment/Salary (full or part-time); Janssen Pharmaceutica.

Poster

478. Ion Channels

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 478.12/B8

Topic: B.04. Ion Channels

Support: CONACyT grants 167052 and 169835

VIEP-BUAP

Title: α-dendrotoxin inhibits the ASIC current in dorsal root ganglion neurons from rat

Authors: *E. SOTO1, A. BÁEZ2, E. SALCEDA2, M. FLÓ3, M. GRAÑA4, C. FERNÁNDEZ5, R. VEGA2;
1Univ. Autonoma De Puebla, Puebla Pue, Mexico; 2Inst. de Fisiologia, Univ. Autónoma de Puebla, Puebla, Mexico; 3Fac. of Chem., Univ. de la República (UDELAR), Montevideo, Uruguay; 4Inst. Pasteur de Montevideo, Uruguay; 5Inst. Pasteur de Montevideo, Uruguay
Abstract: Dendrotoxins are a group of peptide toxins purified from the venom of several mamba snakes. α-dendrotoxin (α-DTx, from the Eastern green mamba Dendroaspis angusticeps) is a well-known blocker of voltage-gated K+ channels and specifically of Kv1.1, Kv1.2 and Kv1.6. In this work we show that α-DTx inhibited the ASIC currents in DRG neurons (IC50 = 0.8 µM) when continuously perfused during 25 s (including a 5 s pulse to pH 6.1), but not when co-applied with the pH drop. Additionally, we show that α-DTx abolished a transient component of the outward current that, in some experiments, appeared immediately after the end of the acid pulse, suggesting that Na+ activated K+ current is involved in this outward current.

Dendrotoxins are basic proteins with a net positive charge at neutral pH. When analyzed in detail, the potassium channel-blocking site of α-DTx and related toxins is formed by residues from the N-terminus and the beta-turn region of the Kunitz domain. Other compounds with known inhibitory action on ASICs are characterized by a strong positive charge at physiological pH. For example, PhcrTx1 has a net charge of around +5.0 at pH 7.4, and a similar charge has been observed for compounds such as aminoglycosides and FMRFamide-related peptides. A cationic domain on the surface of α-DTx may play an important role in the interaction of the toxin with some functional domains of the ASIC channels. A dual action like the one we have found for α-DTx has been reported for other peptide toxins previously considered to be selective. For example, APETx2, a peptide that reversibly inhibits homotrimeric ASIC3, was found to also target Nav1.2, Nav1.6 and Nav1.8-related currents. Our work shows that α-DTx also targets ASICs in the nanomolar range, thus constituting a new potential tool for the study of these channels. The discovery of molecules capable of modulating ASICs may contribute to better understand their function, and provide molecular backbones to design drugs with therapeutic potential. In any case, α-DTx should no longer be considered a selective K+ channel toxin given that it inhibits ASICs and Kv with IC50 in the nM range. Furthermore, our data indicate that many results already in the literature should be re-evaluated taking into account this new finding.

Disclosures: E. Soto: None. A. Báez: None. E. Salceda: C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); CONACyT grant 169835. M. Fló: None. M. Graña: None. C. Fernández: None. R. Vega: None.

Poster

478. Ion Channels

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 478.13/B9

Topic: B.02. Ligand-Gated Ion Channels

Support: American Heart Association (12BGIA8820001)
Title: Identification of a novel allosteric modulator of acid-sensing ion channel 3

Authors: *A. AGHARKAR¹, E. B. GONZALES¹,²,³;

Abstract: Acid-sensing ion channels (ASICs) are sodium selective channels that belong to the ENaC/DEG family of ion channels. They are sensitive to changes in extracellular pH and are expressed in both the central and peripheral nervous system. There are multiple ASIC subtypes that are involved in different pathophysiological conditions, including neurodegeneration, and most recently, epilepsy. Crystal structure of chicken ASIC1 revealed that functional ASIC is a trimer with large extracellular domain that can interact with variety of ligands, and the focus of research has been to identify ASIC antagonists. The ASIC3 channel subtype is primarily expressed in DRG neurons and is involved in pain sensation, but may activate GABAergic interneurons. ASIC3 is modulated by nonproton ligands like 2-guanidine-4-methylquinazoline (GMQ) and agmatine. We have identified a guanidine compound with a different molecule structure than GMQ that allosterically modulates ASIC3. Here we characterize this guanidine ligand using whole-cell patch clamp electrophysiology and ASIC3 transfected CHO-K1 cells. We found that the ligand is able to activate ASIC3 channel at physiological pH of 7.4, similar to GMQ. Furthermore, the guanidine ligand alters low pH current and delays desensitization, indicating that the ligand may be an ASIC3 positive allosteric modulator. The activation by the guanidine ligand was found to be concentration dependent. In the future, we will determine the effect of the guanidine ligand on the ASIC3 window current and identify potential binding sites within ASIC3.

Disclosures: A. Agharkar: None. E.B. Gonzales: None.
**Authors:** *G. HA*, K. SONG, H. KWAK, J. LEE, C. LEE, E. CHEONG;

1Yonsei Univ., Seoul, Korea, Republic of; 2Korea Inst. of Sci. and Technol., Seoul, Korea, Republic of

**Abstract:** In central nervous system (CNS), afterhyperpolarization (AHP) activated after single or multiple action potentials in neurons plays an important role in the modulation of neuronal excitability by limiting firing frequency and thus in the generation of spike-frequency adaptation. Previous studies have often categorized AHP into three groups according to its kinetics: fast AHP (fAHP), medium AHP (mAHP), and slow AHP (sAHP). Many of them have attributed mAHP to calcium-activated potassium channels, such as small-conductance (SK) and large-conductance (BK) calcium-activated potassium channels. We previously reported that calcium-activated AHP currents in thalamocortical (TC) neurons were mediated by multiple components including apamin-sensitive SK channels and niflumic acid-sensitive anion channels whose molecular identity was unknown. Here we investigated the calcium-activated anion channels mediating AHP in TC neurons. The analysis of the reversal potential of anion AHP currents whose amplitude exceeded SK currents indicated that their conduction is mediated by chloride channels. Calcium-activated chloride channels (CaCCs) are involved in many physiological phenomena, but the functional expression and properties of them in CNS have rarely been reported. We characterized the electrophysiological properties of CaCCs in TC neurons and investigated its molecular identity.

**Disclosures:** G. Ha: None. K. Song: None. H. Kwak: None. J. Lee: None. C. Lee: None. E. Cheong: None.

**Poster**

**478. Ion Channels**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 478.15/B11

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

**Support:** Alberta Prion Research Institute Grant

- Alzheimer's Society of Canada Doctoral Scholarship
- Alberta Innovates Health Solutions MD/PhD Studentship

**Title:** Amyloid beta modulates opening of pannexin-1 channels during hypoxia
Ischemic stroke is a condition during which blood flow is reduced in the brain, resulting in downstream hypoxia and cell death. Under low oxygen conditions, there is excessive neurotransmission and impaired neurotransmitter reuptake to astrocytes due to a loss of energy substrates (O2 and ATP). This results in excess glutamate receptor stimulation, anoxic depolarizations (aDPs) and excitotoxicity. The aDP is well characterized, and pannexin-1 channels (Panx1) have been demonstrated to be an important contributing factor to ionic dysregulation during the aDP. Stroke survivors are at risk of developing neurodegenerative disorders such as Alzheimer’s disease (AD). While the reason for this is not fully defined, it has been reported that brain hypoxia leads to increased production of the pathological form of the protein amyloid beta (Aβ). Aβ is aggregated into neuronal plaques in patients with AD, and oligomeric Aβ is thought to disrupt synaptic activity and initiate inflammation pathways, contributing to the common symptoms of the disease. Interestingly, little is known about the physiological role of Aβ, and less still is known of the reason behind its increased production during hypoxia. Our overall hypothesis is that physiological concentrations of Aβ can modulate responses to hypoxia. Using whole-cell patch clamp electrophysiology in pyramidal neurons of the CA1 region of the rat hippocampus, the aDP was assayed using low oxygen conditions. The results of this study have demonstrated that low concentrations of exogenous Aβ significantly attenuate the anoxic depolarization, and may be interacting with Panx1 in order to mediate this effect.

Abstract: Calcium signals are core transducers and regulators in many calcium-dependent signaling cascades. Cytoplasmic calcium signals are generated from intracellular calcium release and external calcium influx from plasma membrane Ca\(^{2+}\)-permeable channels. Neurons are endowed with a large repertoire of ion channels, receptors, transporters and plasma membrane Ca\(^{2+}\)-ATPase that work together to regulate Ca\(^{2+}\) homeostasis. Although voltage-gated calcium channels (VGCCs) and ligand-gated cation channels were thought to be the primary channels involved in Ca\(^{2+}\) homeostasis in neurons, we have demonstrated that store-operated calcium channels (SOCs) are also important in mediating Ca\(^{2+}\) influx in the majority of dorsal horn neurons. Interestingly, we found that a small population of dorsal horn neurons did not have SOCE. This finding raises a question of which type of neurons express SOCs. To answer this question, we took advantage of a transgenic mouse expressing GFP driven by the GAD67 promoter to examine STIM1 expression in GAD67-GFP neurons from cultures and slices using immunohistochemistry. Our results showed that the majority of GABAergic neurons do not express STIM1. To determine whether SOCE is altered in GAD67-GFP neurons, we performed calcium imaging recordings in neurons from cultures and slices prepared from GAD67-GFP mice, and measured calcium release and SOCE induced by 1 µM thapsigargin (a Ca\(^{2+}\)-ATPase inhibitor). Consistent with the STIM1 immunostaining result, both calcium release and SOCE were much smaller in GAD67-GFP neurons than in non-GFP neurons. We then performed patch-clamp recordings to compare voltage-gated calcium currents (I\(_{\text{Ca}}\)) in both GAD67-GFP and non-GAD67-GFP neurons from cultures and spinal cord slices. There was no significant difference of low and high voltage-activated I\(_{\text{Ca}}\) between these two groups of neurons presumably due to large functional diversity of VGCCs in dorsal horn neurons. To further determine the cell types of SOC-expressing neurons, we took advantage of the cell type-specific expression of A-type of potassium channels which are mainly functional in excitatory neurons, and recorded A-type currents (I\(_{A}\)) and SOC currents in the same neurons. The majority of I\(_{A}\)-expressing neurons had SOC currents while non-IA-expressing neurons had no or very small SOC currents, suggesting that SOCs are mainly functional in excitatory dorsal horn neurons. Our results indicate that inhibitory and excitatory neurons have different calcium signals, which may underlie functional differences between these two groups of neurons.


Poster

478. Ion Channels

Location: Hall A
Title: STIMs and Orai1 are responsible for mGluR1/5-mediated ERK activation in spinal dorsal horn neurons


Abstract: Store-operated Calcium Channels (SOCs) are highly Ca2+-permeable cation channels that are intimately involved in the calcium influx pathway. Our previous study demonstrated that SOCs are expressed in dorsal horn neurons and STIM1/2 and Orai1 are responsible for SOCE entry (SOCE). Importantly, we found that inhibition of SOCE attenuates pain hypersensitivity. However, how SOCs are involved in the pain process remains elusive. Our recent findings indicated that inhibition of SOCE reduced spinal ERK activation in collagen-induced inflammatory pain, suggesting that ERK is a downstream target of SOCE. We therefore investigated potential upstream activators of SOCs. SOCs can be activated by the release of Ca2+ from intracellular stores, a process that occurs via activation of the inositol triphosphate (IP3) receptor. It is well known that activation of mGluR1/5 results in production of IP3, which triggers the release of Ca2+ from ER. We hypothesized that activation of mGluR1/5 can lead to SOCE in dorsal horn neurons. We performed Ca2+ imaging and patch-clamp recording to determine the role of SOCs in mGluR1/5 activation and subsequent activation of ERK.

Application of 100 µM Dihydroxyphenylglycine (DHPG) induced an initial transient calcium response, followed by a sustained phase in ~83% of total neurons. Pretreatment with 3µM YM-58483 (a SOCE inhibitor) had no effect on the initial calcium response, however, almost completely blocked the sustained response. DHPG-induced current was also blocked by YM-58483. To confirm that these events were mediated by SOC proteins, STIM1/2 and Orai1 were knocked down by transfection of individual siRNA. Orai1 knockdown caused a marked decrease in sustained Ca2+ increases, while STIM1 and STIM2 knockdown significantly attenuated sustained Ca2+ increases. DHPG-induced phosphorylation of ERK was also blocked by SOC inhibitors and by knockdown of SOC proteins. Together, our data suggests that mGluR1/5 activation leads to SOCE through SOCs, and contribute to mGluR1/5-induced ERK activation. Our findings reveal a novel link of SOCs to mGluR1/5 and to their downstream effectors in dorsal horn neurons. Currently, we are determining whether mGluR1/5 activation leads to translocation of STIM1 and STIM2 to further confirm activation of SOCs by mGluR1/5.

**Title:** Optimal sampling rate and anti-aliasing filter position for the detection of high frequency oscillations (HFOs)

**Authors:** *S. GLISKE, W. C. STACEY;* 
Univ. of Michigan, Ann Arbor, MI

**Abstract:** High frequency oscillations (HFOs) are becoming a well-established biomarker of epileptic tissue. However, translation of this biomarker to clinical settings requires overcoming both scientific and technical challenges. One technical concern is the unknown required and optimal sampling rate and anti-aliasing filter position used when recording HFOs with intracranial EEG. Manufacturing companies are encouraging hospitals and clinics to replace their EEG recording equipment with newer models featuring faster sampling rates, but it is not yet known how high of a sampling rate is actually clinically useful. This study addresses that need using deidentified data acquired from 17 patients undergoing intra-operative monitoring for epilepsy surgery. The intra-cranial EEG data were acquired at greater than 20 kHz, then initially down-sampled to 5 kHz with an anti-aliasing filter position at 2 kHz. A standard set of HFO and artifact detections were determined from the data. HFOs were additionally detected with the data down-sampled to lower sampling rates (500 Hz to 2.5 kHz) or with an additional anti-aliasing filter applied (filter positions from 100 Hz to 1 kHz). HFO detections coincident with detected artifacts were redacted. The number of HFOs detected for each sampling rate and/or filter position were compared with the standard set of HFOs detected at 5 kHz--over 1 million HFOs. Many results were as expected, with the number of detected HFOs at or above 90% for anti-aliasing filter settings above 500 Hz or sampling rates above 2 kHz. Settings below these values resulted in less than 30% of the HFO detections, with the exception of 1 kHz sampling rate still having 70% of the HFO detections. These results were found to be frequency dependent. For example, for HFOs with peak frequency above 150 Hz, a sampling rate of 2.5 kHz already drops the number of HFOs to only 30%. Despite the dramatic changes in HFO detection sensitivity,
only minor changes were observed regarding the clinical correlation of HFOs with epileptogenic tissue.

Disclosures: S. Gliske: None. W.C. Stacey: None.

Poster

479. Oscillations and Synchrony: EEG Studies

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#/Poster#: 479.02/B15

Topic: B.09. Network Interactions

Support: Office of the Dean, Univ. Tennessee HSC (DHH)

iRISE Pilot grant, Univ. Tennessee HSC (DHH, ACP, JWW)

CRCNS Grant NSF/DMS-13-11165

Title: Hilbert analysis of the relation between respiration and LFP/ECoG

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Abstract: Aim: Our goal is to analyze the frequency content of the relationship between the respiratory rhythm and local field potentials (LFPs) and electrocorticography (ECoG) signals. Introduction: Previous studies in mice indicate the presence of a statistically significant correlation between respiration and spike activity or LFPs (Ito et al., 2014). It is of great interest to analyze the robustness of such correlation in intracranial experiments with animals and in human cortical data. Of particular significance is the reliable demonstration that the measured correlation has cognitive relevance and it is not simply a manifestation of movement artifacts. Methods: The experimental approach and the obtained data in mice are described in (Ito et al., 2014); for human ECoG methodology, see (Wheless et al., 2009). The present study determines the amplitude and phase of analytic signals evaluated using Hilbert transformation, which has been widely used for cognitive monitoring and for brain imaging involving fMRI, MEG, ECoG, and EEG data (for a review, see Freeman et al., 2013). We conducted a systematic study of ECoG signals of patients at rest with a sliding frequency window of width 5Hz through theta, alpha, beta, and gamma bands (Kozma et al., 2012). We calculated the instantaneous frequency
of the ECoG signals and correlated it with the respiratory channel. Results: Our study indicates significant correlation between respiration and the instantaneous frequency of ECoG channels in the theta, alpha, and beta frequency bands. However, no clear correlation has been observed between respiration and gamma-band ECoG. Experimental results obtained by recording of human ECoG have been compared with LFPs from mouse. Conclusions: We observe a significant correlation between respiration and ECoG/LFP data, especially in the theta, alpha, and beta frequency bands, while no correlation has been detected at gamma frequencies above approx. 40 Hz. These results point to the importance of respiration on cortical neurodynamics, which cannot be attributed entirely to movement or other external artifacts. Hierarchical models of cortical neurodynamics provide theoretical support to such conclusions (Kozma et al., 2015).


Abstract: Breathing is a basic rhythm of life, whose main purpose it is to secure oxygen supply for the body. Moreover, the respiratory rhythm and the depth of respiratory movements strongly influence cognitive and emotional states. For example, focusing on deep, slow breathing is a common strategy in anger management, meditation and general stress relief (e.g. 1,2). How respiration modifies cognitive and emotional states is currently unknown. We recently reported that respiration modulates rhythmic neuronal activity in the somatosensory whisker barrel cortex of awake mice and that olfactory bulb activity was the main driving force behind these respiration-locked oscillations 3. Here we ask if respiration modulates neuronal activity in other non-olfactory areas of the mouse brain and whether a similar phenomenon exists in humans. We recorded spike and local field potential (LFP) activity in mouse motor, visual and prefrontal cortices, as well as in the hippocampus. We used cross-correlation analysis and circular statistics to determine whether LFP and spike activity were significantly modulated in phase with respiration. In all areas of the mouse brain tested, we found that LFPs at the vast majority of recording sites (>80%) were significantly modulated by respiration. The number of single units where spike activity was significantly modulated by respiration varied between 10 and 32%, depending on the cortical area. The highest proportion of respiration-related single units were found in the trunk portion of the somatosensory cortex and the lowest proportion in the whisker motor cortex. To test whether respiration also influences neuronal activity in the human brain, we analyzed electrocorticography (ECoG) recordings obtained from patients with intractable epilepsy being considered for surgery. Cross-correlation analysis of ECoG and respiration signals from two patients revealed significant correlations between respiration and breathing at several cortical locations. We suggest that rhythmic cortical activity in mice and humans is directly modulated by respiration, most likely via respiration locked sensory inputs, and that this modulation may be the basis for respiratory modulation of cognitive and emotional states.


Poster

479. Oscillations and Synchrony: EEG Studies

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 479.04/B17
Title: Relations between alpha power and the stability of motion-induced blindness

Authors: *H.-M. SUN¹, M. INYUTINA², R. VANRULLEN²,³, C.-T. WU⁴,⁵;
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Abstract: Motion-induced blindness (MIB) refers to a phenomenon in which a static target spontaneously disappears and reappears into consciousness when superimposed on a global moving pattern, despite the fact that it is constantly present in the display. The MIB paradigm has been used as a powerful tool to study visual awareness since it can produce conscious as well as unconscious perception within the same task. However, the neurophysiological correlates of the perceptual target disappearance during MIB remain unclear. Therefore, in the present study, we examined the dynamics of brain oscillations associated with the perceptual target disappearance due to MIB, which would help us to better understand the neural process that distinguishes the neural representation of a perceptually suppressed stimulus from the conscious representation of the same stimulus. Twenty-two participants performed a MIB task while their EEG was recorded. During the task, participants reported the perceptual disappearance and reappearance of a dot target (the MIB condition) by pressing and releasing a response key. We also included a control condition, which simulated the MIB condition with physical disappearance and reappearance of the dot target. The duration of the target disappearance in the control condition was based on the actual target disappearance durations recorded from the MIB condition. The average time between a physical disappearance and the key press in the control condition was used to estimate the onset time of the illusionary target disappearance in the MIB condition. We then tested whether power fluctuations in different frequency bands before the estimated MIB onset would predict the length of MIB. Our results showed a positive correlation across participants between alpha power before the estimated MIB onset and MIB duration ($r = 0.45, p = 0.04$), indicating that participants with stronger alpha power were also the participants who experienced longer MIB. However, no such correlations were found between the power activity in other frequency bands (e.g., delta, theta, beta, gamma) and the length of MIB. Therefore, the results suggest that alpha dynamics plays an important role in the changes in perceptual state and may help shape our visual experience.

Disclosures: H. Sun: None. M. Inyutina: None. R. VanRullen: None. C. Wu: None.
Title: Enhanced thalamic GABAAR-mediated spill-over inhibition promotes electrocortical signatures associated with the induction of NREM sleep and anesthetic-induced loss-of-consciousness

Authors: *L. MESBAH-OSKUI¹, R. L. HORNER²; ¹Med., ²Med. and Physiol., Univ. of Toronto, Toronto, ON, Canada

Abstract: Modulation of thalamic GABAergic signaling can trigger state-associated changes in electrocortical activity. There are three types of GABA₃ receptor (GABA₃R)-mediated inhibition: tonic (i.e., extrasynaptic), phasic (i.e., synaptic), and spill-over which requires both synaptic and extrasynaptic GABA₃Rs. Importantly, the alterations in thalamic activity elicited by the general anesthetic etomidate require both synaptic and extrasynaptic GABA₃Rs in vitro. Here we test two hypotheses in vivo: 1) enhanced thalamic spill-over inhibition elicits changes in electrocortical activity that resemble those elicited by etomidate, such as increased 8-12Hz and 12-30Hz activity, decreased 1-4Hz activity, and increased spindle-like oscillations; and 2) thalamic T-type Ca²⁺ channels, which promote 1-4Hz signaling, do not mediate the changes in electrocortical activity elicited by enhanced spill-over inhibition. Microperfusion of the extrasynaptic GABA₃R positive allosteric modulator DS2 (100uM), which promotes spill-over inhibition, into the ventrobasal complex of the thalamus elicited changes in electrocortical activity in wild-type mice (n = 9), but not in mice lacking GABA₃Rs (n = 8). During NREM sleep, DS2: (i) increased 8-12Hz and 12-30Hz power (both p < 0.006), (ii) decreased 1-4Hz power (p < 0.009), and (iii) increased spindle-like oscillations (p < 0.001) in wild-type mice. Blockade of T-type Ca²⁺ channels with TTAP2 (300uM) did not affect the changes in electrocortical activity elicited by either etomidate (30uM; n = 7) or DS2 (n = 9) at the thalamus (all p > 0.180). Both DS2 and etomidate at the thalamus were associated with an increase in the power of transient electrocortical signatures that have been associated with transitions into both NREM sleep and anesthetic-induced loss-of-consciousness. Specifically, DS2 and etomidate in...
wild-type mice enhanced the transient increase in 14-20Hz power seen during transitions into NREM sleep (both \( p < 0.03 \)). Microperfusion of DS2 or etomidate into the thalamus was further associated with increased time spent in NREM sleep in wild-type mice (both \( p < 0.02 \)). Importantly, the effects identified with DS2 were not recapitulated with THIP (50uM), a direct agonist of GABA\(_A\)Rs, at the thalamus. Additionally, unlike both DS2 and etomidate, the effects elicited by THIP required T-type Ca\(^{2+}\) channel activation (\( n = 9; \) all \( p < 0.05 \)). Collectively, these results implicate enhanced thalamic spill-over inhibition in mediating the electrocortical signatures that are associated with the induction of NREM sleep and anesthetic-induced loss-of-consciousness.

Disclosures: L. Mesbah-Oskui: None. R.L. Horner: None.

Poster

479. Oscillations and Synchrony: EEG Studies

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 479.06/B19

Topic: B.09. Network Interactions

Title: Resting-state EEG oscillatory connectivity between nodes of dorsal visual network as correlate of performance on embedded vs. ambiguous figure tasks in high functioning autism

Authors: *I. SOLIS\(^1\), O. TREGIAK\(^1\), C. BOUCHARD\(^1\), S. MEYER\(^1\), V. LUCE\(^1\), J. M. STEPHEN\(^1\), K. R. CIESIELSKI\(^1,3\);
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Abstract: Objectives. Children with autism spectrum disorder (ASD) show reduced mental flexibility, unusual repetitive behaviors, narrow interests and resistance to change, yet, they may demonstrate superior performance in the analysis of complex visual stimuli using local-details strategy. Neuroimaging findings relate the first to a failure in top-down executive inhibitory control of irrelevant interference, the second to bottom-up sensory processing and lateral inhibition. We hypothesize that reduced mental flexibility and inhibitory abnormalities in a child with ASD may be associated with low integrity of the Dorsal Visual Network (DVN), and in particular it’s long-range dorsal frontal-parietal executive component, while the basic sensory occipital-parietal local network may remain relatively preserved. Methods. Children with ASD, ages 6-16, and matched healthy controls participated. We evaluated integrity of DVN components by examining the amplitude modulation and coherence of EEG resting state (rsEEG
protocol: 4 min fixation on a fine cross). Correlative analysis of Alpha band (8-13Hz) oscillations among the frontal-parietal and occipital parietal nodes was calculated. To test mental flexibility an Ambiguous Figure Test (AFT) and neuropsychological tests were used; for basic inhibitory control on a sensory level, the Embedded Figures Task (EFT) was used. The rsEEG, computing on the level of sensors included: 30sec alpha-band time windows; rsEEG alpha-power using custom script EEGLAB; average alpha coherence in regional clusters by custom MATLAB; Pearson’s correlations. Results. Children with ASD showed: poor accuracy/long time of figure-reversal in AFT (p<0.03), which strongly correlated (r=0.82; p<0.005) with low verbal proficiency. No group differences in accuracy or time of discrimination of figures in EFT were found. Increased rsEEG alpha synchronization indicate a significantly stronger connectivity between coupled frontal-parietal oscillators in the control as compared to ASD group (p<0.02), suggesting an incomplete development of this component of DVN in children with ASD. The correlative values for occipital-parietal oscillators present a similar pattern of connectivity in ASD and Controls. Conclusion. In agreement with prior studies, long-range rsEEG alpha connectivity among frontal and parietal nodes of DVN was significantly reduced in children with ASD. The coherence between occipital-parietal nodes was, however, comparable in both groups. This pattern of connectivity was correlated to low accuracy of reversal figure in AFT and low verbal proficiency.


Poster

479. Oscillations and Synchrony: EEG Studies

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 479.07/B20

Topic: B.09. Network Interactions

Support: Supported by intramural research funds from Furman University

Title: Use of photic stimulation to manipulate resting-state brain activity: a pilot study using high-density eeg

Authors: P. Hickey1, M. Whitmire1, *E. J. Wamsley2;
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Abstract: Memory consolidation has been shown to benefit from short periods of waking rest, but the mechanism underlying this effect remain unknown. During eyes-closed rest, the
prevailing EEG rhythm is alpha (8-12 Hz). In this study, we aimed to experimentally manipulate resting-state alpha activity using photic stimulation, and to test the effect of this manipulation of verbal memory retention. 12 healthy participants underwent a 64-channel high-density EEG recording. A five minute baseline was recorded, followed by three consecutive 10min photic stimulation phases (10Hz, 25Hz, and a non-rhythmic sham). Each stimulation phase alternated between 30sec “on” periods with stimulation, and 30sec “off” periods when stimulation was paused. Prior to each stimulation, a list of 30 words was encoded and freely recalled. A delayed recall was completed after each stimulation phase. Following artifact rejection, we computed the spectral profile of “on” and “off” phases using a fast Fourier transform. 10 Hz stimulation significantly increased power in the 9-11Hz alpha range in occipital (p=0.04), central (p=.03), and frontal (p=.05) regions of interest (“on” vs. “off” comparison). In all regions, enhancement of alpha power (“on”/”off” ratio) was significantly greater during 10Hz stimulation, relative to sham (all p-values <.01). Interestingly, the alpha power at baseline was correlated with alpha enhancement during stimulation (p=0.009). Power was also significantly enhanced at the harmonics of the fundamental frequency, including 9-11 Hz (p=0.04), 19-21 Hz (p=0.01), 29-31 Hz (p=0.01), and 39-41 Hz (p=0.07) (stimulation vs. sham). The topography of the enhancement at harmonics became increasingly focused and more anterior at higher frequencies. In comparison, in the 25 Hz condition, there was no significant effect of stimulation. These observations indicate that photic stimulation is a successful way to both increase a target oscillation during eyes-closed rest, and to strongly affecting the harmonics of the fundamental frequency. Additionally, the magnitude of the effect appears to be dependent on the strength of the endogenous alpha rhythm. There were no statistically significant effects of stimulation on memory consolidation. However, the design of this proof-of-concept study may not have been optimal to detect this effect. In the future we hope to use photic stimulation to further explore the effect of resting-state EEG oscillations on memory consolidation.


Poster

479. Oscillations and Synchrony: EEG Studies

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 479.08/B21

Topic: B.09. Network Interactions

Support: NIH DP2OD006454

CIHR Doctoral Fellowship
Title: Cortical network dynamics of hallucinatory and dissociative states induced by ketamine

Abstract: The brain can generate a range of dissociative and hallucinatory states, both naturally during sleep, and pathologically due to disease or drug use. One drug that can induce these states is ketamine, which primarily acts as an N-methyl-D-aspartate (NMDA) antagonist. Ketamine is used clinically for analgesia and general anesthesia, and has recently been used as a treatment for major depressive disorder. At the high doses used for general anesthesia, ketamine produces unconsciousness and immobility. At lower doses used for analgesia, ketamine causes a dissociative state, in which patients may report feeling disconnected from their bodies and their environment and experience visual and auditory hallucinations, and low-dose ketamine has also been used as a research model of schizophrenia. We aimed to study the neural dynamics that occur during the ketamine-induced dissociative state. We recorded intracranial electrocorticography from four patients with medically intractable epilepsy undergoing an infusion of ketamine prior to clinically indicated surgery. Patients received 0.5 mg/kg ketamine over a 14 minute period, during which time they responded to auditory stimuli presented every 3.5-4.5 seconds. At the end of the infusion period, patients completed an abbreviated version of the Clinician-Administered Dissociative States Scale (CADSS). We found that all four patients entered a dissociative state, as measured by high scores on the CADSS scale. Widespread changes in cortical dynamics accompanied this change in state, most noticeably a strong increase in gamma (~30-70 Hz) power across frontal and temporal electrode sites. Low-frequency (<4 Hz) power underwent variable changes across cortex, and alpha (10-14 Hz) power declined in medial frontal contacts. In contrast, dynamics in the hippocampus changed very little during this state, suggesting that neocortex is more strongly affected by ketamine during the dissociative state. This regional heterogeneity suggests that the effects of low-dose ketamine occur within specific cortical circuits, and that altered dynamics in these circuits may contribute to the dissociative state reported during ketamine administration.

Poster

479. Oscillations and Synchrony: EEG Studies

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 479.09/B22

Topic: B.09. Network Interactions

Title: Modulation of high-frequency oscillations and beta coherence in striato-cortico-limbic circuits following repeated sub-anesthetic ketamine exposure

Authors: *T. YE*, M. J. BARTLETT, J.-P. WIEGAND, M. SCHMIT, S. J. SHERMAN, T. FALK, S. L. COWEN;
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Abstract: Ketamine is a common anesthetic that, when administered as an infusion at sub-anesthetic doses, has been shown in the clinic to be effective for the treatment of chronic pain and treatment-resistant depression. Recent data also show that a low-dose ketamine infusion paradigm did lead to long-term reduction of L-DOPA-induced dyskinesia (LID) in a rat model (20 mg/kg for 10 hrs) and Parkinson’s disease patient case studies (0.15 - 0.3 mg/kg/hr for 72 hrs). Although the mechanisms underlying the anesthetic and medical applications of ketamine are unknown, data from recently published reports suggest that high-frequency oscillations (HFO) (> 100 Hz) and beta-band oscillations (13 - 30 Hz) in the striatum, hippocampus, and cortex are involved. While the effects of single-dose ketamine administration on oscillatory activity has been studied, little is known about the effect of repeated exposure, even less of how ketamine alters coordinated activity between the cortex, striatum, and hippocampus. In the present experiment, we explored these questions by repeated administration of sub-anesthetic doses of ketamine (20 mg/kg) to awake freely behaving rats implanted with arrays of electrodes that targeted the hippocampus, dorsal and ventral striatum, somatosensory cortex, and motor cortex. We recorded over a 10 hr period and administered ketamine (i.p.) once every 2 hrs while rats resided in their home cage (total of five injections). Our preliminary findings indicate that ketamine induces strong HFO (~135 Hz) and beta-band coherence between the ventral striatum and the CA3 region of the hippocampus. Furthermore, HFO coherence increased with repeated injection while beta-band coherence decreased. When the 2nd and final injections were compared, ketamine resulted in a 133% increase in HFO coherence and a 20% reduction in beta-band coherence between the ventral striatum and CA3. Similarly, ketamine induces HFO and beta-band coherence between motor cortex and dorsolateral striatum, with a 6% increase in HFO coherence and a 2% reduction in beta-band coherence between 2nd and final injections. The gradual increase in coherence and in HFO induced by successive injection of ketamine suggest
that ketamine induces lasting (hours) changes in limbic-striatal and striato-cortical connectivity. Such lasting changes may underlie the established improvement following ketamine treatment in patients experiencing chronic pain or depression and the similar benefit in the treatment of LID reported by our group.


**Poster**

**479. Oscillations and Synchrony: EEG Studies**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program/#Poster#: 479.10/B23

**Topic:** B.09. Network Interactions

**Title:** Exploring relationships between eeg theta/beta ratios, empathy, reward sensitivity, and anxiety

**Authors:** *S. GARRETT-RUFFIN, E. HERRING;* Psychology, Bowling Green State Univ., Bowling Green, OH

**Abstract:** By establishing relationships between neural oscillations and psychological disorders, researchers may be able to establish diagnostic markers and objective measures of treatment outcomes. For example, a well-established finding is increased resting electroencephalogram (EEG) slow wave/fast wave ratios in people with attention deficit hyperactivity disorder (ADHD). EEG theta (4-8 Hz)/beta (13-30 Hz) ratios are now used as part of the diagnosis for attention deficit hyperactivity disorder (ADHD). Larger T/B ratios are thought to reflect cortical under arousal, which is consistent with the under arousal theory of ADHD. The purpose of this study was to extend research on T/B ratios to affective traits. Similar to ADHD, affective traits such as empathy, reward sensitivity and anxiety are related to cortical arousal states which lead to a number of predictions: 1) T/B ratios would be positively correlated with empathy deficits; 2) T/B ratios would be positively correlated with reward sensitivity; and 3) T/B ratios would be negatively correlated with anxiety, with larger T/B ratios linked to less anxiety. The sample was composed of 30 right-handed female undergraduate students aged 20-27, with no history of neurological disease. EEG theta waves (4-8 Hz) and beta waves (13-30 Hz) were recorded under four conditions (eyes open, eyes closed, reading and listening) to yield a T/B ratio composite score. Active recordings were taken along the midline at two sites: Fz and Cz. Next, participants completed a number of affective measures: the Interpersonal Reactivity Index (IRI), the Behavioral Inhibition and Behavioral Activation (BIS/BAS) Scale and the State Trait-Anxiety
Inventory (STAI-t). The IRI consists of subscales designed to measure the cognitive (perspective and fantasy) and the emotional (empathy and personal distress) dimensions of empathy. The BIS/BAS consists of a scale measuring behavioral inhibition (BIS) and behavioral activation (BAS), comprised of the subscales BAS reward, BAS drive, and BAS fun seeking. The STAI-t provides a single anxiety trait score. All of the hypotheses were supported by the research. As predicted, T/B ratios were positively correlated with empathy deficits, specifically the personal distress subscale of Interpersonal Reactivity Index (Davis, 1980). Additionally, T/B ratios were positively correlated with reward sensitivity. Finally, T/B ratios were negatively correlated with anxiety, with larger T/B ratios linked to less anxiety. Future research will involve exploring the effects of empathy manipulations on T/B ratios.

**Disclosures:** S. Garrett-Ruffin: None. E. Herring: None.

**Poster**

479. Oscillations and Synchrony: EEG Studies

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 479.11/B24

**Topic:** B.09. Network Interactions

**Support:** Kjell and Märta Bejers Foundation

Swedish Research Council

Brazilian Agencies CAPES/CNPq

**Title:** OLM interneurons promote theta activity the ventral hippocampus

**Authors:** *R. N. LEAO*¹, ², S. MIKULOVIC², E. RESTREPO², S. PUPE², K. KULLANDER², A. TORT¹;

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**Abstract:** Theta oscillations in the dorsal hippocampus are described as one of the most regular rhythms of the brain. This 4-12 Hz have been associated with multiple behaviors, especially with movement. On the other hand, theta activity in the ventral hippocampus has been implicated in emotions related behavior. It has been hypothesised that specific GABAergic interneuron subtypes play differential roles in driving hippocampal oscillations. Although different populations of hippocampal interneurons fire preferentially to specific phases of theta, phase-locking firing itself does not prove a causal role in theta generation. We found a specific subtype of oriens lacunosum-moleculare (OLM) interneurons expressing Chrna2 receptor differentially
distributed along the dorso-ventral hippocampal axis. Using optogenetic tools in anesthetised and freely behaving animals, we found that activation of this population induce prominent theta activity in the ventral but not in the dorsal hippocampus. Interestingly, the induced theta rhythm was not correlated with animals' movements. In treadmill experiments, we found that rhythmical optical stimulation of OLM neurons can either increase or decrease the coherence between dorsal and ventral hippocampus depending on the position of the optical fibre within the dorsoventral axis of the hippocampus. Taken together, our results provide the first evidence of a single morphologically defined cell population that in a network including pyramidal cells causally drives ventral hippocampal theta oscillations.


Poster

479. Oscillations and Synchrony: EEG Studies

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 479.12/B25

Topic: B.09. Network Interactions

Support: NIH-NINDS NS030549

Coelho Endowment to I.M.

Title: Entrainment of parvalbumin+ interneurons \textit{in vivo} depends on movement rather than theta oscillations

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Abstract: Impaired parvalbumin positive (PV+) interneuron (IN) function and gamma oscillatory activity have been identified both in patients and animal models of several neurological and neuropsychiatric disorders. Furthermore, recent findings show that optogenetic activation of PV+ INs can lasting improve cognitive deficits observed in an animal model of schizophrenia. However, currently there are no data relating the ability of PV+ INs for orchestrating gamma oscillations to the behavioral/brain states in a healthy subject. In this study we addressed this missing link by optogenetically stimulating PV+ INs in the hippocampal CA1 region in awake head-fixed PV Cre x Ai27 mice. In agreement with previous findings, 40 Hz blue (473 nm) laser light stimulation induced the appearance of a 40 Hz local field potential
(LFP) component recorded in the hippocampal CA1 pyramidal cell layer. We also found that the magnitude of the evoked 40 Hz oscillations was significantly higher when the animal was moving, i.e., when theta oscillations were present in the LFP recordings, compared to epochs when the animal was motionless or no theta oscillations were present. The measured amplitudes of the LFP oscillations at 40 Hz were as follows (in mV units, n=6 mice): without laser stimulation no theta vs theta: 11.8 ± 0.6, 14.5 ± 1.6, p=0.16; during laser stimulation no theta vs theta: 17.0 ± 1.5, 24.2 ± 2.9, p=0.005 (2-way repeated ANOVA with Bonferroni correction). Because theta oscillations and movement of the animals are highly correlated (Pearson's correlation coefficient: 0.75, p<0.0001, n=6) the question arose whether it is the presence of theta activity or movement that regulates the ability of PV+ INs to generate gamma oscillations. To answer this question we carried out the same PV+ IN stimulation protocols following the injection of the local anesthetic lidocaine into the medial septum. Medial septal lidocaine injections reliably eliminated theta oscillations even when the animal was moving. Surprisingly, we found that when theta oscillations were blocked, the magnitude of the light-evoked 40 Hz oscillations in the LFP showed a robust dependency on the animal’s mobility (n=4 mice, in the presence of lidocaine, without laser stimulation no move vs move: 8.6 ± 1.0, 9.8 ± 1.4, p=0.14; with laser stimulation no move vs move: 12.1 ± 1.1, 19.6 ± 2.3, p=0.008, 2-way repeated ANOVA with Bonferroni correction). Our findings are consistent with the idea that the ability of PV+ INs to orchestrate gamma oscillations is closely linked to the animal’s mobility and is independent of the presence of theta oscillations.

Disclosures: A.M. Barth: None. I. Mody: None.

Poster

479. Oscillations and Synchrony: EEG Studies

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 479.13/B26

Topic: B.09. Network Interactions

Title: Phase synchronization analysis of K-complex and Sleep spindles using ensemble measure in healthy subjects

Authors: *C. S. NAYAK¹, N. MARIYAPPA², P. D. PRASAD³, K. K. MAJUMDAR⁴, T. KANDAVEL³, A. B. TALY², S. SINHA²;
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Abstract: Background: K-complexes (KC) coupled with Sleep spindles (SS) are believed to arise from the synchronized, sleep-specific, burst-pause firing of large number of neurons making up the thalamo-cortical loop. This study aims to assess the change in phase synchronization indices (SI) during the peri-KC spindle period (PKP) on scalp electroencephalographic (EEG) recordings during sleep. Methods: Ten healthy subjects (mean age: 23.7±3.06 years; M: F = 5:5) were subjected to overnight polysomnography using 8-channel EEG. Eight KCs associated with SS (KC-SS) were visually identified in each participant and subjected to phase synchronization analysis using multichannel measure across all the 8 channels based on Frobenius norm (taking three equal time windows before, during and after KC-SS, each equal to the length of that KC-SS). Changes in SI were assessed for delta (δ; 0.5-3Hz), theta (θ; 4-7Hz), alpha (α; 8-12Hz) and beta (β; 13-30Hz) frequency bands. The mean ± standard deviation of SI at the three time windows during PKP were calculated for various frequency bands, and compared using repeated measures ANOVA (RM-ANOVA) followed by pair-wise comparisons (p≤0.05). Results: RM-ANOVA revealed significant changes in SI in PKP for the δ band, along with a significant increase in SI during and after KC-SS as compared to before KC-SS (p<0.001). There was also a trend towards higher SI during KC-SS as compared to after KC-SS, although not statistically significant (p=0.636). However, other frequency bands (θ, α and β) did not show significant changes in SI during PKP. Conclusion: This is the first study that has used ensemble measure to assess the cortical phase synchronization during PKP. The findings of this study support the previous view of quasi-synchronous firing during KC and SS, and may also suggest the persistence of such synchrony for some period after KC and SS have occurred. These findings may have implications in understanding the role played by phase synchronization in influencing the occurrence of epileptic activity during sleep.


Poster 479. Oscillations and Synchrony: EEG Studies

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 479.14/B27
Title: P1 in the somatosensory evoked EEG response solely reflects neural excitation: a concurrent EEG and LFP study

Abstract: A proportional balance between neural excitation and inhibition is a fundamental principle that underlies normal brain function, and an imbalance has been implicated in a number of disorders such as autism, schizophrenia and Alzheimer’s disease (Rubenstein et al. 2003; Palop et al. 2007; Lewis et al. 2012). Electroencephalography (EEG) can be used as a diagnosis tool for identifying the presence of brain related disease and disorders. Unfortunately, it is unclear how to isolate neural excitation from inhibition in the evoked EEG response. However, single neuron recordings show co-tuning of excitatory and inhibitory synaptic activity, and inhibition lagging excitation by several milliseconds (Wehr and Zador 2003, Okun and Lampl 2008). If this temporal lag can be observed at a population level the contributions of inhibition and excitation to the evoked EEG response can be isolated. We previously demonstrated, using a rodent model, that local field potentials (LFPs) could be decomposed into neural excitatory and inhibitory components (Bruyns-Haylett et al. 2014). Here we extend our previous findings using concurrent EEG and LFP recordings. The balance between neural excitation and inhibition was pharmacologically manipulated by micro-injecting subconvulsive concentrations of bicuculline (10µM), a competitive gamma-aminobutyric acid (GABAA) receptor antagonist, in the barrel cortex of anaesthetised rodents. We investigated changes in LFPs and EEG before and after infusion in response to electrical stimulation of the contra-lateral whisker pad. We found no statistically significant difference in the temporal dynamics of P1 in EEG responses between the control condition and after bicuculline infusion. This indicates that P1 may be solely associated with excitatory post-synaptic activity of the local neural population, and that P1 reflects the level of neural excitation in a cortical network. Furthermore, magnitude of the first negative peak (N1) increased after bicuculline infusion, suggesting that N1 is modulated by the degree of inhibition within a cortical network and related to the onset of inhibitory post-synaptic activity. References Bruyns-Haylett M, et al. (2014). Washington, DC: Program No. 535.15/I121, Somatosensory: Local Cortical Circuits, Society for Neuroscience, 2014. Lewis, David A., et al. Trends in neurosciences 35.1 (2012): 57-67. Okun, M. and Lampl, I., 2008. Nat. Neurosci. 11, 535-537.

Poster

479. Oscillations and Synchrony: EEG Studies

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 479.15/B28

Topic: B.09. Network Interactions

Support: NIH R01 DC004290-14

Title: Efficient and comprehensive measurement of interregional phase-amplitude coupling and characterization through tensor decomposition

Authors: *C. K. KOVACH*¹, P. E. GANDER², A. E. RHONE¹, M. J. SUTTERER¹, H. KAWAKAMI¹, R. ADOLPHS³, M. A. HOWARD, III¹;
¹Dept. of Neurosurg., ²Univ. of Iowa Hosp. and Clinics, Iowa City, IA; ³Humanities and Social Sci., Caltech, Pasadena, CA

Abstract: Cross-frequency coupling in the form of dependence between amplitude at one frequency and the phase of ongoing oscillations at a lower frequency (Phase-amplitude coupling, PAC) is a commonly observed feature of local field potentials, thought to reflect the time-regulation of excitability in neuronal populations. PAC has most commonly been considered within local populations, though recent work shows that it may also reflect long-range interactions across widely separated regions, with amplitude at one recording location linked asymmetrically to phase at another. Interregional PAC may therefore serve as a probe of large-scale network interactions, but comprehensive measurement of interregional PAC within large multi-channel recording arrays is computationally challenging. Equally challenging is the interpretation of the resulting sizable multi-way array of interactions. We describe an efficient and spectrally comprehensive approach to interregional PAC measurement and demonstrate its application to human ECoG data from four epilepsy patients under a variety of experimental conditions. Using tensor decomposition, we identify large-scale patterns of PAC associated with each condition and show that the time-domain representation of the coupling spectrum as an impulse-response function may provide crucial details on the nature of the coupling. The approach is validated in its ability to blindly recover an expected dependence between an EMG signal contaminant arising from the extraocular muscles and cortical post-saccadic evoked responses, showing that ocular EMG contamination may be a source of spurious PAC with a
characteristic spectral signature. In all four patients, PAC occurred between phase at a range of
narrow-band frequencies intervals centered from < 1 Hz to high beta range (<30 Hz), coupled to
a broad range of amplitude bands from 30 to 200 Hz. Resting with eyes closed was associated
with coupling between high-gamma amplitude within both early visual and auditory cortices and
alpha-band phase across a widely distributed network, which included dorsolateral prefrontal
cortex and temporal pole. Video watching was associated with more focal intra-regional alpha-
band coupling in early sensory cortices nested in delta-band fluctuations occurring over anterior
temporal lobe and inferior frontal gyrus. These results confirm the presence of inter-regional
PAC with narrow coupling spectra and show its potential for elucidating the organization of
functional networks in the brain.


**Poster**

**479. Oscillations and Synchrony: EEG Studies**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program/#Poster#: 479.16/B29

**Topic:** B.09. Network Interactions

**Support:** Sara Page Mayo Endowment for Pediatric Pain Research & Education (LC; CBB)

- NIH Grant R01-GM104948 (ENB; SK)
- NIH Grant DP2-OD006454 (PLP)

**Title:** Postnatal development of spatial and temporal EEG characteristics of anesthetic state in infants 0-6 months

**Authors:** *L. CORNELISSEN¹, S.-E. KIM², P. L. PURDON³, E. N. BROWN², C. B. BERDE⁴;


**Abstract:** BACKGROUND: In adults, electroencephalogram (EEG) characteristics observed
during general anesthesia-induced unconsciousness consist of widespread slow activity (<1Hz),
and frontal predominant alpha activity (8-12Hz) and coherence [1]. Evidence shows that EEG-
based indices developed and used in adults provide inaccurate assessments of anesthetic states in children, particularly in infants. The aim of this study was to characterize the spatial and temporal EEG characteristics in infants aged 0-6 months during the awake state, and maintenance of, and emergence from sevoflurane general anesthesia administered for routine surgical care. METHODS: Continuous 32-channel EEG recordings were performed during the awake state, and during maintenance of a Surgical State of Anesthesia (MOSSA) and emergence-from sevoflurane general anesthesia in 30 infants aged 0 to 6 months postnatal age. We analyzed EEG recordings with multi-taper-spectral and coherence methods, and video recordings of body movement. We compared the power spectrum and coherence within and between infants aged 0 to 3 months (n=11) and 4 to 6 months (n=19) during the awake state, MOSSA, and emergence (immediately after first body movement was observed) using the bootstrap approach. Coherence was estimated between frontal channels F7 and F8. RESULTS: During MOSSA: 1) all infants had slow (0.1 to 1Hz) and delta (1 to 4Hz) oscillations across the entire scalp; 2) theta (4 to 8Hz) and alpha (8 to 12 Hz) oscillations emerged from 4 months of age; 3) across all frequencies, the frontal power in the EEG was significantly greater in the 4 to 6 month-old infants compared to the 0 to 3 month-old infants; and 4) unlike in adults, all 0 to 6 month-old infants showed an absence of frontal alpha coherence. We also show that in the awake state, and during emergence (immediately after first body movement), power in the alpha, theta and beta bands was significantly lower compared to the MOSSA state in 4 to 6 month-old infants. CONCLUSION: We show that infants aged 0 to 6 months of age have markedly different EEG patterns from each other, and from adults under general anesthesia. These differences are likely due to differences in brain development and help explain why EEG-based indices provide inaccurate measures of anesthetic states in infants less than 4 months. Furthermore, they suggest the types of studies and analyses that are required to develop neurophysiologic-based strategies for anesthetic state monitoring in pediatric patients.


Disclosures:  L. Cornelissen: None. S. Kim: None. P.L. Purdon: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patents pending on brain monitoring during general anesthesia and sedation, and have a patent licensing agreement with Masimo Corporation. E.N. Brown: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patents pending on brain monitoring during general anesthesia and sedation, and have a patent licensing agreement with Masimo Corporation. C.B. Berde: None.

Poster

479. Oscillations and Synchrony: EEG Studies

Location: Hall A
**Title:** Optimizing repetitive brain stimulation using direct electrical recordings in human neocortex

**Authors:** *M. FINI*, C. KELLER, C. HONEY, F. LADO, A. MEHTA; 

**Abstract:** Keywords: brain stimulation, ECoG, cortico-cortical evoked potential (CCEP), high gamma envelope (HGP)

**Background:** Brain stimulation is increasingly used to treat psychiatric diseases yet the generators and underlying sustained network changes are not well-understood. We sought to elucidate these effects by applying electrical stimulation and recording directly to the human neocortex in epilepsy patients undergoing monitoring for surgery.

**Methods:** Five patients undergoing surgical evaluation were enrolled. After electrode implantation, electrical responses were recorded in three conditions, with order counterbalanced across patients: 10Hz (4s train, 26s rest, 10min total); 1Hz (13 min train); sham stimulation. All conditions included 800 pulses. Current was adjusted with 4mA being used in most patients.

**Results:** Stimulation pulses within the 10 Hz train induced both positive and negative voltage deflections in evoked CCEP amplitudes across various brain areas. Significant changes to the evoked responses were measured 2-15 minutes post stimulation with all evoked responses returning to baseline within 15 minutes. Modulated areas were correlated with distance from the stimulation site, strength of pre-stimulation CCEP amplitude, and resting HGP correlation with the stimulation site. Areas showing significant amplitude change exhibited a prestimulation CCEP amplitude 2.5 +/- 0.7 fold that of non-modulated areas and were located 60 +/- 5% closer to the stimulation site. These effects were not observed for 1Hz or sham stimulation. Using a machine learning algorithm, modulated regions could be predicted with 77% accuracy using only pre-stimulus CCEP amplitude.

**Conclusions:** Repetitive stimulation elicited changes that were predictable, short-acting, within 4.8cm of the stimulation site, and selective for the 10 Hz pulse rate. These results suggest the timing of pulses during repetitive stimulation can be modified to induce stronger and longer-lasting changes in brain networks. Both positive and negative CCEP deflections were observed, indicating that the 10 Hz stimulation significantly affects both excitatory and inhibitory networks. Modulated areas fell within a few centimeters of the stimulation site and, exhibited a strong pre-stimulation CCEP amplitude and resting HGP correlation with the stimulation site. These factors can be used to predict areas where significant change will occur. Future work will include improving statistical methods, including other stimulation parameters.
(1 Hz, 20 Hz, theta burst), time-frequency analysis of CCEPs for HGP analysis, and multi-day stimulation to determine long term local network changes.

**Disclosures:** M. Fini: None. C. Keller: A. Employment/Salary (full or part-time); Albert Einstein College of Medicine. C. Honey: A. Employment/Salary (full or part-time); Montefiore Medical Center, Bronx, NY. F. Lado: A. Employment/Salary (full or part-time); Albert Einstein College of Medicine. A. Mehta: A. Employment/Salary (full or part-time); North Shore Hospital.

**Poster**

**479. Oscillations and Synchrony: EEG Studies**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#: 479.18/B31**

**Topic:** B.09. Network Interactions

**Support:** Department of Anesthesiology at the University of Michigan

NIH Grant RO1GM098578

**Title:** Conditions for explosive synchronization in human brain networks during general anesthesia

**Authors:** M. KIM¹, G. A. MASHOUR²,⁴, *U. LEE³,⁴

¹Physics, POSTECH, Pohang, Korea, Republic of; ²Anesthesiol. and Neurosci. Grad. Program, ³Anesthesiol., Univ. of Michigan Med. Sch., Ann Arbor, MI; ⁴Ctr. for Consciousness Sci., Ann Arbor, MI

**Abstract:** Background: Although there are a number of common neural correlates of sleep, anesthesia and coma, the recovery profiles of these states are radically different. To understand the reversibility of unconsciousness at a network level, we studied the topological and dynamical properties of brain networks in conscious and lightly anesthetized states. Methods: Multi-channel electroencephalograms (EEG) from seven healthy subjects and the weighted Phase Lag Index (w-PLI), a phase locking measure, were used to construct the functional networks across waking, transitional, unconscious and recovery states. We investigated the topological and dynamical properties that have been suggested recently as conditions for explosive synchronization (ES) in general networks: (1) disassortativity and (2) the frequency difference of coupled nodes. Furthermore, the suppressive rule, an inequality relationship for ES between local frequency difference and global network properties, was assessed to find out the local contribution for
conditions of ES. The brain region with the largest contribution to ES was identified for each EEG epoch during anesthesia to investigate the regional contribution across states. Results: Anesthesia disrupted the hub structure in the posterior parietal and occipital lobes, and the peak frequency of alpha power shifted toward higher frequency at the frontal region. The high disassortativity value of the waking state was significantly reduced and the frequency difference of coupled nodes was significantly increased in the unconscious state (Disassortativity: waking (Mean ± SD, -0.3590 ± 0.0538) vs. unconscious (Mean ± SD, -0.1458 ± 0.0408), P < 0.001, frequency difference: waking (Mean ± SD, 0.0043 ± 0.0021) vs. unconscious (Mean ± SD, 0.0149 ± 0.0051), P < 0.005, using repeated 1-way ANOVA with tukey’s multi-comparison test). All values returned to baseline with the recovery of consciousness. The number of local nodes that facilitate ES with a small perturbation rapidly increased after induction. The trigger nodes for ES in the brain networks were variable across regions over time. Conclusion: Anesthesia suppresses the capacity of information integration by reconfiguring the brain network. However, the increase in conditions for ES suggests that the lightly anesthetized brain network is still in a strongly resilient state and that a small perturbation has the potential to trigger an explosive transition back to the normal network state. Further assessing ES conditions in brain networks during deep general anesthesia, sleep and coma may provide insight into the variable recovery profiles of these unconsciousness states.

Disclosures: M. Kim: None. G.A. Mashour: None. U. Lee: None.

Poster

479. Oscillations and Synchrony: EEG Studies

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 479.19/B32

Topic: B.09. Network Interactions

Support: CIHR

Title: Effect of sleep restriction on EEG activity during deep isoflurane anesthesia

Authors: T. MARIAM, R. TADAVARTY, *P. J. SOJA; Univ. British Columbia, Vancouver, BC, Canada

Abstract: Restricted sleep and its consequences are endemic to society. While sleep and anesthesia overlap in their overt characteristics, it is known that the loss of consciousness that occurs following inhalational anesthesia happens much sooner when sleep is restricted. However, few, if any studies have actually examined the effects of sleep deprivation on the
concentration-dependent effects that inhalational anesthetics exert on cortical EEG activity. Accordingly, we employed the phenomenon of EEG burst suppression events (Swank & Watson, 1949) as a reliable biomarker representing a deep surgical plane of anesthesia to address this issue. Male Sprague-Dawley rats (360-380 g) were divided in three groups (I-control, II-sleep deprived for 9 h, and III-recovery for 48 h, n=6/group). Rats in each group were initially anesthetized with isoflurane (ISO) using an induction chamber, the animals’ trachea were then intubated and their head mounted in a stereotaxic frame. Cortical EEG (S1) was recorded bilaterally using stainless screw electrodes. The concentration of anesthetic (ISO%) was initially adjusted to produce a stable baseline EEG that consisted of large-amplitude slow-wave activity over a 30 min period. ISO was then increased in relative 0.5% steps of 0.5, 1.0, 1.5, and 2.0 while the number of burst events/min were automatically detected and quantified by a computer subroutine. In group I, the number of burst events/min at relative 0.5, 1.0, 1.5 and 2.0 ISO% measured 20.5 ± 1.5, 9.8 ± 2.3, 3.5 ± 0.7, and 0, respectively. In group II, the dose-related suppression of burst events by the same increasing relative ISO% was shifted to the right. The number of bursts events increased proportionally at the same 0.5, 1.0, 1.5 and 2.0 ISO% and measured 25.9 ± 1.9, 20.4 ± 1.8, 10.2 ± 2.0 and 1.5 ± 0.5, respectively. The dose-related ISO suppression of burst events in group III animals returned toward group I levels and measured 17.9 ± 2.5, 10.3 ± 1.8, 4.7 ± 0.7 and 0, respectively. Tukey’s test following repeated measured two-way ANOVA indicated that the number of bursts/min at ISO% 0.5, 1 and 1.5 was significantly increased (p<0.05) in group II rats compared to group I rats, which reversed back to control level in group III rats. On the basis that burst suppression events reflect an inactivated brain with reduced metabolism, our findings on burst suppression events across the three groups of animals would suggest that sleep deprivation per se may enhance cerebral metabolism. Notwithstanding the obvious clinical relevance, our findings may provide a basis for more careful design of future animal studies investigating sleep and/or anesthetic mechanisms.


Poster

479. Oscillations and Synchrony: EEG Studies

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 479.20/B33

Topic: B.09. Network Interactions

Support: TR01-GM104948
DP2-OD006454
Title: Thalamocortical synchronization during propofol-induced unconsciousness

Authors: *F. J. FLORES*1,2,4, K. HARTNACK1, A. B. FATH4, S. E. KIM2, N. KOPELL6, M. A. WILSON3, E. N. BROWN1, P. L. PURDON1;

Abstract: In humans, loss of consciousness induced by the anesthetic propofol is accompanied by the appearance of frontally coherent alpha (8-12 Hz) oscillation in the EEG (Purdon et al. 2013). Modelling studies have suggested that these alpha oscillations arise from thalamocortical synchronization due to the potentiating effect of propofol on GABAergic synaptic transmission (Ching et al. 2010). To test this hypothesis, we recorded local field potentials simultaneously from pre-frontal cortex and midline thalamic nuclei in awake, freely behaving rats. To mimic the slow increase in inhibitory conductance used as a tuning parameter in the model, we administered propofol as a slow intravenous infusion whose rate was derived from an allometric pharmacokinetic model. We estimated loss of consciousness by assessing loss of the righting reflex. Beta (13-16 Hz) oscillations associated with behavioral hyperactivity appeared in both the thalamic and cortical sites before loss of consciousness. With loss of consciousness the beta oscillations transitioned to coherent alpha oscillations between the thalamus and cortex. Following loss of consciousness delta (1-4 Hz) and alpha oscillations appeared in the pre-frontal cortex. During this period, thalamocortical coherence in the delta range is very high. The thalamic alpha oscillations are weaker, but alpha coherence is still present. Thalamocortical alpha coherence regains power prior to recovery of consciousness. Our results show that in rodents propofol produces the same oscillatory patterns observed in human EEG recordings. Our findings support the hypothesis that propofol-induced frontal alpha oscillations result from coherent thalamocortical activity that occurs at the point of loss of consciousness.


Poster

479. Oscillations and Synchrony: EEG Studies

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM
Title: Intracranial alpha dynamics and correlates of anteriorization during propofol general anesthesia


Abstract: Stereotyped signatures of general anesthesia in the electroencephalogram (EEG) have been recently studied and include dynamics now known to be caused by disruption of GABA-ergic thalamocortical networks driven by propofol, a GABA agonist. Among the key features of the EEG during propofol general anesthesia is the change in distribution of alpha power from posterior in the awake brain to anterior in the unconscious brain. The intracranial correlates of these dynamics are not well understood. We studied neural recordings from 14 patients with medically intractable epilepsy who had been previously implanted with intracranial depth and electrocorticography (ECoG) electrodes as general anesthesia was induced with propofol while performing a behavioral task. We demonstrate an anterior and medial shift of coherent alpha activity using frequency domain principle component analysis. We also show that anteriorization of alpha power is associated with two distinct phenomena. First, alpha power decreases in posterior sensory cortices (auditory and visual) as waking alpha oscillations are disrupted and slow oscillations increase during loss of consciousness. Second, de novo alpha rhythms begin in frontal and midline structures, including cingulate cortex and frontal white matter. In addition, we find that in a subset of posterior brain regions with alpha rhythms before loss of consciousness, task-related alpha dynamics in sensorimotor and auditory regions were disrupted during anesthetic induction. Regionally distinct alpha behavior in the frontal and occipital cortices suggests that thalamocortical connections to each brain region generate separate components of the alpha dynamics observed during induction of propofol general anesthesia. Therefore, intracranial recordings provide rich, region-specific detail to alpha dynamics previous studied in surface EEG.

intellectual property rights/patent holder, excluding diversified mutual funds); Masimo Corporation. F. Consulting Fees (e.g., advisory boards); Masimo Corporation.

Poster

479. Oscillations and Synchrony: EEG Studies

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 479.22/B35

Topic: B.09. Network Interactions

Support: TR01-GM104948

DP2-OD006454

Title: Evidence for slow oscillations and coherent theta and gamma oscillations during ketamine-induced altered arousal

Authors: *O. AKEJU\textsuperscript{1}, F. J. FLORES\textsuperscript{1}, K. J. PAVONE\textsuperscript{1}, M. A. WILSON\textsuperscript{2}, P. L. PURDON\textsuperscript{1}, E. N. BROWN\textsuperscript{1};
\textsuperscript{1}Massachusetts Gen. Hosp., Boston, MA; \textsuperscript{2}Massachusetts Inst. of Technol., Cambridge, MA

Abstract: Ketamine is an N-methyl-D-aspartate antagonist that is used as an anesthetic, a research model for schizophrenia, and a fast acting treatment for major depressive disorder. At low doses, ketamine produces a dissociative state characterized by hallucinations, altered sensory perception, and analgesia, while at higher doses it induces a state of unconsciousness appropriate for general anesthesia. In contrast to other general anesthetic drugs, ketamine does not depress cerebral glucose utilization or cerebral blood flow, and it is associated with gamma oscillations in the electroencephalogram (EEG). The mechanisms underlying these contrasting states of consciousness and assorted neural effects are the subject of intense interest in neuroscience. We recorded the EEG during ketamine-induced anesthesia (2mg/kg). Immediately after general anesthesia induction, we observed a “slow-gamma” pattern (slow oscillations alternating with gamma oscillations; \( n = 12 \)) that eventually transitioned to a “gamma stable” pattern. Both the slow-gamma and the gamma stable patterns were associated with increased theta oscillations (4-8 Hz) and decreased beta/gamma oscillations (10-20 Hz). The slow-gamma pattern was also associated with large amplitude slow/delta (0.1-4 Hz) and beta/gamma (20-50 Hz) oscillations. When we compared the coherence patterns of both ketamine patterns, we found that the coherence structure was similar for all frequencies studied (0.1-50 Hz). We next recorded ketamine-induced (150mg/kg) local field potentials (LFP) and single unit activity in the prefrontal cortex of Sprague Dawley rats. Single unit recordings revealed that gamma bursts
were associated with increased firing rate of pyramidal neurons, while slow oscillation bursts were associated with increased firing rate of inhibitory neurons. Our results suggest the following: (1) Active inhibitory circuits in the cortex are instrumental for the ketamine-induced slow oscillations (2) Ketamine mediated increase in gamma oscillations are likely secondary to the effects of ketamine on basket interneurons (reduced inhibitory tone) resulting in pyramidal neuron excitation (3) The slow-gamma pattern is not a state of neuronal burst suppression (4) Ketamine-induced theta and beta/gamma oscillations are coherent and likely function to reduce the dimensionality of normal cortical information processing networks. These findings will facilitate a more principled brain-state monitoring paradigm in clinical settings. Additionally, they may also aid research in the treatment of depression, pain disorders and schizophrenia.

Disclosures: O. Akeju: None. F.J. Flores: None. K.J. Pavone: None. M.A. Wilson: None. P.L. Purdon: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Masimo. F. Consulting Fees (e.g., advisory boards); Masimo. E.N. Brown: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Masimo. F. Consulting Fees (e.g., advisory boards); Masimo.

Poster

479. Oscillations and Synchrony: EEG Studies

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 479.23/B36

Topic: B.09. Network Interactions

Support: CIHR

Title: Effect of very deep isoflurane anesthesia on hippocampal plasticity

Authors: *R. TADAVARTY, T. MARIAM, P. SOJA;
The Fac. of Pharmaceut. Sci., The Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Cortical EEG activity beyond the isoelectric line of very deep coma induced by inhalational anesthetics is poorly understood. Specific patterns of activity emerge in the electroencephalogram (EEG), as the dose of anesthetic is increased. In animal studies, isoflurane (ISO) is a commonly used volatile anesthetic. When ISO concentrations are increased, EEG activity changes from large-amplitude slow waves to recurring periods of bursts, followed by isoelectric EEG. The number of burst events proportionally decrease with increasing concentration of ISO (1-3%), beyond which the EEG consists mainly of randomly occurring
single or bursts of fast large-amplitude spikes. Additionally, at higher ISO concentrations, EEG activity between large-amplitude spike events is dominated by rhythmic low-amplitude oscillations (Anesth Analg 1994; 79:52-7, PLoS One 2013; 8(9):e75257). Although, hippocampus has been suggested as one source of these large-amplitude cortical spike waves during very deep ISO coma, the functional consequences within the hippocampal networks during this state are not known. The present study examined in-vivo effects of prolonged exposure to deep ISO (5%) on long-term depression (LTD) of excitatory post-synaptic transmission in the CA1 region of the hippocampus in-vitro. Male SD rats (400-600g) were initially anesthetized in an induction chamber, their trachea intubated and head mounted in a stereotaxic frame. Cortical EEG was recorded using stainless steel screw electrodes positioned in S1 bilaterally. Hippocampal slices were then prepared from either control (n=5) or test (n=5) animals exposed to deep ISO (5%) for at least 3 h. Field excitatory postsynaptic potentials (fEPSPs) were evoked at 0.05 Hz by stimulating the CA3 afferents, and recorded from the apical dendritic layer of CA1 pyramidal neurons. While a low-frequency stimulation (LFS; 1 Hz, 1200 pulses), reliably induced LTD in the control group, the time-course and extent of LTD was significantly altered in rats exposed to deep ISO (5%). Amplitudes of fEPSPs recovered to the pre-tetanic baseline levels in the test group in ~ 8 min post-LFS. At 20 min post-tetanus, LTD magnitude was significantly larger (fEPSP amplitudes as a % of pre-tetanic controls, 73.6 ± 5.2, n=5; Student’s t test; p<0.05) in control animals when compared to the test group (109.9 ± 8.0; n=5). LTD’s functional role(s) include among other things, fine tuning of overall excitability of hippocampal and cortical neural networks. The marked suppression of LTD observed in deeply comatose brains of adult animals would likely afford a shift toward excitatory processes, the consequences of which remain to be determined.

Disclosures: R. Tadavarty: None. T. Mariam: None. P. Soja: None.

Poster
479. Oscillations and Synchrony: EEG Studies

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 479.24/B37

Topic: B.09. Network Interactions

Support: VA Career Development Award (JMM)

VA Merit Award (RWM)

NIMH R01 MH0039683 (RWM)
Title: Optogenetic and pharmacological manipulation of cortical excitatory/inhibitory balance: rescuing the effects of acute ketamine on cortical gamma band oscillations

Authors: *J. M. MCNALLY, S. THANKACHAN, R. W. MCCARLEY, R. E. BROWN; VABHS, Harvard Med. Sch., Brockton, MA

Abstract: Gamma band (30-80 Hz) oscillations (GBO) serve as a fundamental mechanism allowing coordination of neuronal activity during various cognitive processes. Abnormalities in GBO have been observed in a number of neuropsychiatric disorders such as schizophrenia (Sz), and appear to arise from dysfunction in the cortical circuitry responsible for generating such activity. It has been hypothesized that impaired NMDA receptor function in cortical interneurons is largely responsible for GBO abnormalities. This NMDA hypofunction has been suggested to alter the delicate balance in activity between the inhibitory and excitatory components of the cortical circuit (E/I balance), principally through reducing the activity of interneurons which express parvalbumin (PV). Sub-anesthetic doses of NMDA antagonists (e.g. ketamine) effectively model symptoms of Sz in humans and elicit Sz-like GBO and behavioral impairment reminiscent of Sz symptoms in animals. However, methods to correct ketamine and Sz-induced GBO abnormalities are lacking. Here, utilizing both optogenetic and pharmacological techniques, we investigated several strategies to upregulate PV interneuron activity, restore cortical E/I balance, and potentially rescue GBO. First, we focused on cortically projecting basal forebrain (BF) GABAergic PV neurons, which target cortical PV interneurons and modulate cortical activation/GBO (see Kim et al., 2015, PNAS 112(11):3535). Optogenetic inhibition of BF PV neurons in freely behaving mice (n=4) partially rescued the elevated spontaneous GBO activity elicited by acute ketamine (30 mg/kg). Further, tonic optogenetic excitation of BF PV neurons (n=2) induced an elevation in spontaneous GBO by itself. Second, we examined the type 5 metabotropic glutamate receptor (mGluR5), which is expressed by cortical PV neurons, and has been shown to facilitate NMDA receptor function. Studies have shown positive modulators of mGluR5, are capable of alleviating Sz-like behavioral deficits, and may provide a means to rescue Sz-related NMDA hypofunction. Here, using an in vitro slice model of GBO, CDPPB (10-20 µM; n=4), a positive modulator of mGluR5, while having no effect on the GBO alone, partially inhibited the acute potentiation of these oscillations by ketamine (100 µM). Together, these findings suggest that 1) optogenetic manipulation of the activity of BF PV neurons and 2) pharmacological facilitation of mGluR5 activity both may provide effective targets for modulating cortical E/I balance, and restoring normal GBO activity in Sz.


Poster

479. Oscillations and Synchrony: EEG Studies
Title: Visual encoding is gamma phase dependent

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Abstract: Gamma oscillations have been implicated in feature binding at different levels of visual information processing from low-level feature analysis (Gray & Singer, 1989) to conscious perception (Martinovic & Busch, 2011). However, their causal role in visual perception has long been debated. We have developed a new paradigm for testing the causal role of gamma oscillations in the segmentation of stimulus flow. Subjects were instructed to fixate and attend a rapid succession of 8 images of birds and cars (in separate blocks) flashed for 32 ms duration each, in an RSVP paradigm while 128-channel EEG was recorded. Each RSVP trial was followed by a visual recognition task where the participants were asked to identify the initial 8 images from a gallery of 16 images, half of which were new. The selected images were sorted into two groups: correctly recognized stimuli and incorrectly recognized. Failure of recognition could have been due to an encoding failure, a memory retrieval failure, or both. We analyzed the Hilbert transform of EEG of 15 adult subjects during the stimulus encoding at three frequency bands: theta, alpha and gamma (1-8 Hz, 9-15 Hz, 30-60 Hz, respectively). An average oscillation phase profile was computed from the EEG associated with the encoding of correctly recognized stimuli. This average phase profile of correct encoding was compared to the average phase profile of EEG oscillations associated with the failure of encoding, separately for each frequency band. We observed a significant phase difference between the correctly and incorrectly recognized stimuli in gamma band for the bird image dataset, but no similar difference was observed in other frequency bands and data sets. We validated the gamma phase difference in the bird image dataset by re-computing it after shuffling the image-response associations and the difference disappeared, proving that phase difference did not occur by chance. The largest difference was observed at occipital electrode positions, but occipital-frontal and occipital-temporal electrode groups also displayed significant phase differences. The phase difference emerged as early as 70 ms after stimulus onset, consistent with the earliest C1 component of visually evoked potential (Di Russo, Mart'nez, Sereno, Pitzalis, & Hillyard, 2002). This suggests that the encoding-related gamma phase difference could not be induced by the stimulus, but instead, the gamma phase sets a time window optimal for sampling the visual input. Hence, it is
not the stimulus that resets the gamma phase but rather the actual gamma phase that enables the processing of visual information.

**Disclosures:** J. Csatlos: None. S. Linnert: None. E. Kormann: None. Z. Nadasdy: None.

**Poster**

479. Oscillations and Synchrony: EEG Studies

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 479.26/B39

**Topic:** B.09. Network Interactions

**Title:** Intrinsic excitability measures track anti-epileptic drug action and uncover increasing/decreasing excitability over the wake/sleep cycle

**Authors:** *C. MEISEL¹, A. SCHULZE-BONHAGE², D. PLENZ¹;
¹NIMH, Bethesda, MD; ²Univ. of Freiburg, Freiburg, Germany

**Abstract:** Dynamic changes of excitability are relevant in both healthy and pathological cortical network dynamics. This is particularly highlighted by the pathological changes in excitability of cortical tissue commonly underlying the initiation and spread of seizure activity in patients suffering from epilepsy. Accordingly, controlling excitability using anti-epileptic drugs (AED) and monitoring their effect on network excitability is of prime importance for adequate clinical care and treatment. To date, adequate measures of excitability and action of AED have been difficult to identify. Recent insights into normal ongoing activity in humans, non-human primates and cortical *in vitro* preparations have identified measures such as (1) the distribution of clustered synchronized events, as well as (2) entropy and (3) global level of phase synchronization which characterize normal levels of excitability and quantify any deviation therefrom. Here, we explore the usefulness of these measures to quantify cortical excitability in humans using ongoing multi-day intracranial EEG recordings. We report a significant covariation of markers with the level of AED, a post-ictal drop and a characteristic 24-h time course which increases during the day and returns to baseline at night. Our results indicate that excitability in epileptic networks is effectively reduced by AED and suggest the proposed markers as useful candidates to quantify excitability in routine clinical conditions without the need of electrical or magnetic stimulation. The intradian time course of these metrics provides unprecedented evidence in humans for a homeostatic role of sleep: to rebalance cortical excitability.
Figure: Multi-day monitoring of cortical excitability under changing levels of AED. a, Top: changes in the distribution of synchronized events D. Middle: mean (R) and variability (H) of phase synchronization (brown 4-8 Hz, orange 8-12 Hz, green 12-25 Hz). Bottom: AED dosage. b, Differences between days of low and high AED levels for all 8 patients for marker D. c, Differences for R and H. *p≤0.05, and **p≤0.001.

Disclosures: C. Meisel: None. A. Schulze-Bonhage: None. D. Plenz: None.

Poster

479. Oscillations and Synchrony: EEG Studies

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 479.27/B40

Topic: B.09. Network Interactions

Support: NIMH/NIH Intramural Program

Title: Intrinsic frequency biases across the posterior-anterior cortical hierarchy

Authors: *M. S. MELLEM1, A. GHUMAN2, S. WOHLTJEN1, A. MARTIN1; 1NIH, Bethesda, MD; 2Dept. of Neurolog. Surgery, UPMC, Pittsburgh, PA

Abstract: Recent findings in primates suggest that intrinsic periodic spiking activity occurs on different timescales across cortical areas. An organization following the anatomical hierarchy
underlies the timescales such that activity in sensory areas occurs on faster timescales (50-150ms) while activity in higher-order areas occurs on slower timescales (>150ms). It is not yet known if a similar timescale hierarchy is present in human subjects. Additionally, measures in the primate studies have not considered that periodic activity within a brain area can occur at multiple frequencies. We hypothesized that, in humans, intrinsic neural activity in sensory areas would be biased towards higher frequencies (faster timescale) while higher-order areas would be biased towards lower frequencies (slower timescale). To test this, we examined the spectral power in multiple frequency bands across several ROIs from task-independent data using magnetoencephalography (MEG). Neuromagnetic responses were recorded from 13 subjects at 600 Hz using a 275 channel whole-head MEG system while subjects fixated for five minutes. The data was downsampled to 150 Hz and bandpass filtered from 1-50 Hz off-line. Structural MRIs were collected for each subject, dense cortical grids were created with Freesurfer, and the MEG signal was source localized using the minimum norm estimate inverse solution. Timeseries from six hierarchically-organized ROIs (V1, V2, V4v, fusiform gyrus, lateral prefrontal cortex, orbitofrontal cortex) were extracted. Spectral power was calculated for each ROI and normalized by the total power for each ROI. The group-averaged spectra revealed that most of the power was in either a low band (< 2Hz) or the alpha band (8-12Hz). Thus group power was averaged over each band for each ROI for further statistical analysis. A 2x6 ANOVA revealed a Frequency band X ROI interaction (p<0.001), and Spearman’s rank correlation was used to investigate if intrinsic spectral power in these two bands followed the hierarchical ordering. Low frequency power increased from V1 up to Orbitofrontal (rho = 0.94, p<0.05), while alpha power decreased with increasing anatomical hierarchy (rho = -0.89, p<0.05). These results suggest two main conclusions: Not only are higher-order areas biased towards 1-2Hz relative to sensory areas, and sensory areas towards alpha frequency relative to higher-order areas, but that there is a gradient of power across the anatomical hierarchy for both frequency bands. Thus, intrinsic activity within a brain area occurs at multiple timescales with the amount of activity that occurs at fast and slow timescales biased based on the cortical hierarchy.

Disclosures: M.S. Mellem: None. A. Ghuman: None. S. Wohltjen: None. A. Martin: None.

Poster

479. Oscillations and Synchrony: EEG Studies

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 479.28/B41

Topic: B.09. Network Interactions

Support: KAKENHI 10425423
Title: Cooperation and specialization of the bilateral hippocampi in rodents

Authors: *Y. SHINOHARA, A. HOSOYA, H. HIRASE;
RIKEN, Wako, Japan

Abstract: In rodents, the left and right hippocampi are synaptically connected. Accordingly, the ipsi- and contralateral CA3-CA1 projections are termed as the Schaffer and commissural pathways, respectively. Although the existence of such anatomical connections has been known for nearly a century, relatively little is known about their projection topology, molecular composition, and physiology. By retrograde tracing, we characterized the relative contributions of CA3 and CA2 innervation to CA1 stratum oriens (s.o.) or stratum radiatum (s.r.). We find that CA2 predominantly projects to ipsilateral CA1 s.o. and this projection accounts for approximately 10% of the input. The largest source of innervation to CA1 s.o. is the contralateral CA3. By contrast, the ipsilateral projection from CA3 is predominant in CA1 s.r. While the macroscopic connectivity of the interhemispheric CA3-CA1 projection appears to be symmetric, left-right asymmetry is apparent in spine morphology and synaptic molecule composition. We demonstrate that CA1 s.r. spines innervated by the right CA3 have contrasting spine morphology and ionotropic glutamate receptor subunits to those innervated by the left CA3. Finally, we demonstrate that enriched environment (EE) housing enhances the power and synchrony of interhemispheric theta-associated gamma oscillations in CA1 s.r. Interestingly, the gamma power increase is more prominent in the right side. On the other hand, the amplitude and left-right asymmetry of ripple oscillations were not significantly altered. In a separate set of EE rats, spine density increases are manifested in the right CA1 s.r. relative to the left of the same animal. These results suggest that experience influences the wiring and operational dynamics of bilateral hippocampi.

Disclosures: Y. Shinohara: None. A. Hosoya: None. H. Hirase: None.

Poster

479. Oscillations and Synchrony: EEG Studies

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 479.29/B42

Topic: F.01. Human Cognition and Behavior
Title: Exploring the neural basis of the electrophysiological power spectrum

Authors: *R. GAO¹, B. VOYTEK²;
¹Cognitive Sci., UCSD, La Jolla, ; ²Dept. of Cognitive Sci., UCSD, La Jolla, CA

Abstract: The power spectrum of meso- and macro-scale brain electrical recordings in the forms of the local field potential (LFP), electrocorticogram (ECoG), and electroencephalogram (EEG) are often described to be following an inverse power law relationship, given by \( P = AF^{-(x)} \), where \( F \) is frequency, \( P \) is power, and \( A \) and \( x \) are free parameters characterizing the power law. In the log-log domain, this relationship is represented by a linear trend with a negative slope of \(-x\) and a \( y\)-intercept of \( \log A \). This phenomenon has been well documented in empirical data, noting changes in \( A \) and \( x \) during various perceptual and motor tasks. In addition, recent computational models using population-spiking neurons have attributed these parameters to different biological mechanisms. While the power law formulation of the spectrum has proven fruitful, two key observations are unsatisfactorily accounted for. First, there have been reports of an increase in strictly high gamma power (>60Hz), resulting in a curling of the spectrum, without changes in the slope or intercept. Second, rotations of the spectrum resulting from a change in slope are observed to be about a frequency of ~25Hz, instead of 1Hz, which would be the case if only a change in \( x \) were to occur. Here, we argue that a strict inverse power law model is an incomplete description of the underlying processes giving rise to the power spectrum, and propose the addition of an additive term, i.e. \( F = AF^{-(x)} + B \). Furthermore, we postulate that \( B \), a broadband signal akin to white noise, arises from de-correlated (Poissonic) population firing engaged in local computation. Using a Poisson population model, we demonstrate that an increased firing rate leads to both an increase in gamma power (high frequency curling) and a rotation of the spectrum about ~25Hz. In addition, we validate our model by demonstrating an improved fit of the power spectrum derived from human ECoG and rat LFP data. In summary, the new formulation has both explanatory powers over the data and a sound neurophysiological basis, improving our understanding of the power spectrum of electrophysiological data.

Disclosures: R. Gao: None. B. Voytek: None.
Abstract: There is a little known about nuclear lectin features and function in nerve tissue cells. As it has been shown they participate in the protein-protein interaction, which is implemented by protein carbohydrate interaction. We studied rat brain cell nuclear membrane GlcNAc-specific lectins, but nothing was known about the same glycoproteins in calf brain. GlcNAc-specific lectins were obtained by Akhalkatsi et al. from calf (1 - 1,2 years old) brain cell nuclear membrane fraction using Chauveau method without chaotropic and reductive substances. So obtained protein fraction hemagglutination activity showed, that titre is equal 256 and specific activity is 341. From this protein fraction calf brain cell nuclear membrane GlcNAc-specific lectin has been obtained by affinity chromatography with column GlcNAc immobilized on tris-acrylle. After further rechromatography which was obtained by HPLC (Whaters, USA) on tandem columns Protein PAK 300 SW, U-60 showing four fractions with molecular masses 70kD and more: 63,10 and 1,6kD. Rechromatography of all four fractions showed maximum absorption in the ultraviolet area at 208-212nm, which points to the absence or presence of following amino acids: tyrosin and tryptophan in a very low concentration. The exception is the only 1,6kD fraction, which has the second maximum at 280nm. The calf brain cell nuclear membrane GlcNAc-specific lectin is a glycoprotein and contains carbohydrate 2,25 µg/100µg protein measured by Kolb and Kamishnikov. Specific activity of these lectins is equal 104,4 and it is thermostabile. In the structure of carbohydrate binding center of calf brain cell nuclear membrane GlcNAc-specific lectins take part Ca²⁺ and Mg²⁺ ions 2,90 µg and 0,7µg/100 µg protein correspondingly, which was showed with the influence different concentration of EDTA and EGTA on the hemagglutination activity. GlcNAc-specific lectins does not contain Mn²⁺ ion. The ions content in calf brain cell nuclear membrane lectins was measured by atomic absorption method.
Title: Intrinsic plasticity accompanies synaptic LTD in Purkinje cells

Authors: *Z. YANG, F. SANTAMARIA;
Biol., UTSA, San Antonio, TX

Abstract: Cerebellar Purkinje cells show activity dependent changes of intrinsic excitability in both behavioral learning and in vitro slice recordings. It is still unknown whether long term depression (LTD) of parallel fiber-Purkinje cell (PF-PC) synapse is associated with modulation of intrinsic membrane properties. We performed whole-cell patch-clamp recordings in slice from 16- to 23-day-old mice. PF-PC LTD was induced in voltage clamp mode by pairing parallel fiber stimulation and somatic depolarization. This protocol generated robust reduction of EPSC of about 40%. Intrinsic properties were recorded in current clamp mode before and after synaptic LTD induction. We found an increase of overall excitability in somatic recording after LTD induction. There was also a parallel decrease of sag ratio and an increase of input resistance at more hyperpolarized potential, indicating a reduction of h-current. We did not observe significant changes in afterhyperpolarization amplitude following action potential trains. The application of HCN channel blocker ZD 7288 occluded the increase of excitability without affecting synaptic LTD. Since there are two components in our LTD induction protocol, we further tested the excitability changes by somatic depolarization or parallel fiber stimulation only, in order to elucidate the stimulation pattern required for this intrinsic plasticity. We found that neither stimulation protocol alone can lead to overall excitability change, indicating the requirement of concomitant somatic depolarization and dendritic stimulation for intrinsic plasticity. Our results show that LTD induction enhances Purkinje cell excitability through downregulation of HCN channels. This non-synaptic plasticity may serve as a homeostatic mechanism and could affect future information processing by Purkinje cells.

Disclosures: Z. Yang: None. F. Santamaria: None.
Neural Oscillators and Activity-Dependent Plasticity of Intrinsic Membrane Properties

Location: Hall A
Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM
Program/#Poster#: 480.03/B45
Topic: B.10. Intrinsic Membrane Properties
Support: European Research Council (FP7/2007-2013)/ERC Grant agreement P261114
National Multiple Sclerosis Society grant (RG 4924A1/1)

Title: Spatiotemporal dynamics of calcium flux in the axonal initial segment

Authors: M. POPOVIC, *M. H. KOLE;
Netherlands Inst. for Neurosci., Amsterdam, Netherlands

Abstract: Vertebrate neurons have developed a complex arrangement of voltage-gated ion channels expressed at high densities in domains of myelinated axons to optimize action potential (AP) initiation, conduction and transmitter release. Although there is a wealth of information about the precise biophysical mechanisms by which calcium (Ca\(^{2+}\)) ions regulate the release of neurotransmitter at the presynaptic boutons, the origin and functional role of Ca\(^{2+}\) in the axon initial segment (AIS) and nodes of Ranvier remains poorly understood. To assess the spatial and temporal dynamics of axonal Ca\(^{2+}\) we filled thick-tufted rodent layer 5 pyramidal neurons with high-affinity 100 µM Oregon Green BAPTA 1 during whole-cell patch-clamp recording and used high-speed epi-fluorescence Ca\(^{2+}\) imaging to measure Ca\(^{2+}\) changes in the axon during subthreshold and suprathreshold membrane potential changes. Ca\(^{2+}\) signals were expressed as fractional fluorescence changes scaled to the dye saturating pulse (80 APs at 400 Hz) to eliminate the effect of different background fluorescence. It was found that excitatory postsynaptic potential (EPSP) like current injections (5 EPSPs at 100 Hz) triggered substantial Ca\(^{2+}\) signals in the axon and spatially confined to both the AIS and the nodes of Ranvier with the time-to-peak matching the depolarization duration, i.e. ~50 ms (n = 30). These subthreshold Ca\(^{2+}\) transients were voltage dependent and completely blocked by 200 µM cadmium. While suprathreshold Ca\(^{2+}\) transients were present in all axonal domains, the highest concentration occurred in a spatially restricted ~5 µm hot spot at the AIS (n = 10). The decay of calcium signal in the hot spot was slower than in the other axonal regions with ~33% increase in the decay time constant. Nimodipine preferentially blocked the subthreshold signals (by ~40%), whereas both Mibebradil and nickel preferentially blocked the suprathreshold signal (by ~50%) suggesting the involvement of voltage-gated L- and T-type Ca\(^{2+}\) channels (n = 6) as the sources of calcium at the AIS during EPSP integration and AP initiation, respectively. These results suggest that Ca\(^{2+}\)...
signals in the AIS are highly compartmentalized and functionally segregated, capable to detect and participate in the integration of the co-incidence of synaptic inputs.

**Disclosures:** M. Popovic: None. M.H. Kole: None.

**Poster**

480. Neural Oscillators and Activity-Dependent Plasticity of Intrinsic Membrane Properties

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 480.04/B46

**Topic:** B.10. Intrinsic Membrane Properties

**Support:** VA Merit Review Grant

**Title:** Regulatory evolution and voltage-gated ion channel expression in squid axon: selection-mutation balance and fitness cliffs

**Authors:** *D. MCKINNON, M. KIM, D. D. MCKINNON, T. MACCARTHY, B. ROSATI; Stony Brook Univ., Stony Brook, NY

**Abstract:** It has been suggested that optimization of either axonal conduction velocity or the energy efficiency of action potential conduction predominates in the selection of voltage gated sodium and potassium channel expression levels in the squid axon. A population genetics model that incorporates the effect of mutation on channel gene regulatory function as well as developmental/phenotypic noise on channel protein expression was used to examine the role of these and other evolutionary forces on the selection of channel expression levels [1]. In this model, the accumulating effects of mutations result in degradation of gene regulatory function, causing channel gene expression to fall to near-zero levels, in the absence of positive selection. In the presence of positive selection, channel expression levels fall to the lowest values consistent with the selection criteria, thereby establishing a selection-mutation balance. The simulation results suggest the following conclusions: 1. Mutation of gene regulatory function will cause voltage-gated channel expression levels to trend towards the lowest levels consistent with any positive selection criteria acting on the system. This process will generally produce an energy efficient system, even in the absence of positive selection for energy efficiency. To convincingly argue that minimization of energy utilization is a critical constraint on the evolution of voltage-gated channel expression levels it is necessary to demonstrate that some other function, that is also important, is compromised in order to maximize energy efficiency, i.e. demonstrate that there are tradeoffs associated with the maximization of energy efficiency. 2. In
many cellular electrophysiological systems, including the squid axon, large regions of channel conductance parameter space can produce similar physiological performance. The inescapable effect of mutations in degrading gene regulatory function means that most of this parameter space will not normally be occupied \textit{in vivo}, in the absence of some significant countervailing positive selection criteria. Genetic variation in gene regulatory function within populations, and developmental/phenotypic noise within individuals, are major causes of individual variation in protein expression levels in most biological systems. These basic genetic/biochemical mechanisms are also likely to produce significant variation in channel expression in electrically excitable cells, without the need to resort to more complex mechanisms, such as activity-dependent homeostasis. [1] Kim et al. (2015) PLOS One. 10(4), e0120785.


Poster

\textbf{480. Neural Oscillators and Activity-Dependent Plasticity of Intrinsic Membrane Properties}

\textbf{Location:} Hall A

\textbf{Time:} Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

\textbf{Program/#Poster#:} 480.05/B47

\textbf{Topic:} B.10. Intrinsic Membrane Properties

\textbf{Support:} IGERT CMMB 0965918

\hspace{1cm} NSF IOS 1354913

\textbf{Title:} Circadian rhythm of redox state in hippocampal CA1 regulates neuronal membrane excitability

\textbf{Authors:} *G. NASERI KOUZEHGARANI$^1$, M. YU$^2$, M. U. GILLETTE$^3$;
\hspace{0.5cm} $^1$Neurosci. Program, $^2$Mol. and Integrative Physiol., $^3$Cell and Developmental Biol., Univ. of Illinois at Urbana-Champaign, Urbana, IL

\textbf{Abstract:} Circadian rhythms regulate many aspects of metabolic homeostasis. At the cellular level, metabolic state manifests as redox state, the ratio of redox-molecule pairs, e.g., glutathione disulfide (GSSG)/glutathione (GSH), that originates from reduction-oxidation reactions. We previously reported a novel model for the interdependency of the transcriptional-translational clock machinery, redox oscillation, and neuronal excitability in the hypothalamic suprachiasmatic nucleus (SCN, Wang \textit{et al.}, \textit{Science} 2012; Gillette & Wang, \textit{Antioxid. Redox}
Signaling, 2014). Does this interrelationship exist in extra-SCN brain regions? Here we focus on the hippocampus, where Per 2 clock gene expression in CA1 pyramidal cell layers undergoes a robust circadian oscillation, 180° out-of-phase with that of the SCN. We first evaluated neuronal excitability of CA1 neurons at various times of day in rat hippocampal brain slices using whole-cell patch-clamp recording. Based on recordings from 69 current-clamped neurons, we observed oscillations in membrane potential (Vm) that varied with circadian time (CT), such that neurons were hyperpolarized during the subjective day (-75.98 ± 1.77 mV, CT 7) and depolarized during the subjective night (-68.84 ± 0.64 mV, CT 14). Average resting Vm in midday (CT 5-7) was significantly more hyperpolarized than during the late day (CT 10-12), early night (CT 13-15), or late night (CT 16-20) (-74.99 ± 1.00, -71.43 ± 0.61, -68.46 ± 0.57, and -69.08 ± 0.85 mV, respectively, p < 0.01). Next, we assessed time-of-day changes in redox state. Glutathiolation, the capacity of proteins to incorporate reduced glutathione, was lowest in hippocampal tissue at CT 14. This corresponds to a more reduced state. Experimental manipulation of redox state rapidly altered membrane excitability. The reducing reagent, glutathione (GSH, 1 mM), depolarized Vm in a reversible manner. Average GSH-induced changes in Vm were dependent on CT and were significantly larger at midday (CT 7) than any other time (p < 0.05). Minimal effects were caused during early night (CT 14). In conclusion, both membrane excitability and redox state undergo circadian oscillations in CA1 neurons in rat hippocampal brain slices. This finding extends observations of coupled non-translational oscillators discovered in the SCN to the hippocampus, and raises questions about molecular and cellular substrates. Insights into these interdependencies are critical to understanding modulatory brain functions organized around the day-night cycle.


Poster

480. Neural Oscillators and Activity-Dependent Plasticity of Intrinsic Membrane Properties

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 480.06/B48

Topic: B.10. Intrinsic Membrane Properties

Support: TR-SFB134 to P.K.

CECAD to P.K.

CONNECT to P.K.
Fellowship of the Cologne Graduate School of Ageing Research to U.C.

**Title:** Plasticity of intrinsic firing patterns in midbrain dopaminergic neurons

**Authors:** *U. COLLIENNE*1,2, S. HESS1,2, M. E. HESS2,3, S. POPOVYCH1,4, S. DAUN-GRUHN1,4, J. C. BRÜNING2,5,6, P. KLOPPENBURG1,2;


**Abstract:** The midbrain dopaminergic neurons constitute an essential part of the reward/hedonic system in mammals. *In vivo* studies with combined electrophysiological recordings and behavioral tests showed that the prediction and detection of rewards can be correlated with three firing patterns of dopaminergic neurons: (1) a single spike pattern, (2) a burst pattern and (3) a hyperpolarized state in which the cell remains silent. *In vitro*, dopaminergic neurons have typically been described to be highly regular pacemakers that generate spikes in low frequencies between 1 and 10 Hz (Brain Res Rev. 2008; 58(2), 314-21). This pacemaker activity is intrinsically generated, since it persists in complete synaptic isolation. However, a subpopulation of dopaminergic neurons in the substantia nigra does not generate pacemaker activity, but fires with an irregular spike pattern. Interestingly, the number of irregularly firing dopaminergic neurons increases during ageing. The difference in the spike pattern between pacemaking and irregularly firing dopaminergic neurons seems to be induced by a change of the calcium-dependent pathways (J Physiol. 2010; 588(10), 1719-35 and J. Neurosci. 2014; 34(28):9310-8).

We investigate age-dependent, metabolic modulation of the intrinsic electrophysiological properties of dopaminergic neurons. In previous studies we showed that insulin application depolarizes the membrane potential and an increases firing rate in dopaminergic neurons. Mice with an insulin receptor knock-out in dopaminergic neurons exhibited an increased body weight, increased fat mass, and hyperphagia (Cell Metab. 2011; 13:720-728). Here we ask if the irregularly firing dopaminergic neurons are also modulated by fuel sensing signals and whether the modulation differs from dopaminergic neurons with pacemaker activity. To address this question we use perforated patch clamp recordings of synaptically isolated dopaminergic neurons in acute brain slices. Acknowledgments: We thank Helmut Wratil for outstanding technical assistance.


Poster
**480. Neural Oscillators and Activity-Dependent Plasticity of Intrinsic Membrane Properties**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 480.07/B49

**Topic:** B.10. Intrinsic Membrane Properties

**Support:** NHRI-EX104-10105NI

\[\text{MOST 103-2320-B-010-041-MY3}\]

**Title:** Axonal action potentials in hippocampal dendrite-targeting interneurons

**Authors:** *J.-Y. WENG, C.-C. LIEN;
Natl. Yang-Ming Univ., Taipei, Taiwan

**Abstract:** Activity-dependent modulation of action potential (AP) waveforms occurs in axons of glutamatergic neurons and contributes to synaptic plasticity. Yet, how AP waveforms are regulated in GABAergic interneuron axons is unknown. Here, we combined whole-cell patch-clamp recording and voltage imaging to investigate APs in a typical dendrite-targeting interneuron, oriens-lacunosum-molecular layer cells (OLM cells) in the rat hippocampal CA1 region. We found that axonal APs in a train had no accommodation in amplitudes and no broadening in AP width compared to APs in soma and dendrites. Pharmacological experiments indicate that Kv1- and Kv3-like channels underlie AP repolarization and prevent use-dependent AP broadening. Although axonal AP waveforms are not dynamically modulated by activity, AP propagation failures in axons were noted. Notably, axonal AP propagation failures can be reduced by the GABAA receptor blocker SR-95531, but enhanced by the GABAA receptor agonist muscimol. Our results indicate that OLM cells are featured by unique axonal AP signaling.

**Disclosures:** J. Weng: None. C. Lien: None.

**Poster**

**480. Neural Oscillators and Activity-Dependent Plasticity of Intrinsic Membrane Properties**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM
Program/#Poster#: 480.08/B50

Topic: B.10. Intrinsic Membrane Properties

Support: Fondecyt 1140700 (MS)
Fondecyt 3150668 (JV)
Fondecyt 1130177 (JA)

Title: Contribution of persistent Na+ current and muscarine-sensitive K+ current to perithreshold theta resonance in CA1 pyramidal neurons

Authors: *J. A. VERA, J. ALCAYAGA, J. BACIGALUPO, M. SANHUEZA;
Univ. De Chile, Santiago, Chile

Abstract: Most neurons from hippocampus and other learning- and memory-related areas have the ability to intrinsically generate subthreshold rhythmic activity at theta frequency (4-10 Hz), which may contribute to the strong theta waves observed during hippocampal-related behaviors like navigation or episodic memory formation. Pyramidal neurons from CA1 area receive theta rhythmic inputs from other brain regions, generating place fields. The way these neurons respond to perithreshold oscillatory stimulation and thus their possibility to translate frequency preference into spiking has been controversial; most evidence favors a non-resonant or integrator-like behavior while other studies suggest a resonant behavior. The ionic currents contributing to perithreshold behavior of pyramidal neurons are the persistent sodium current $I_{NaP}$, a depolarizing fast-activating current that develops above -70 mV and the slower activating, hyperpolarizing muscarine-sensitive K+ current $I_M$, with -60 mV threshold potential. With current- and voltage-clamping we conducted a detailed characterization of perithreshold excitability of CA1 pyramidal neurons under oscillatory stimulation by somatic current injection. These neurons displayed two different perithreshold behaviors: 20% of them expressed resonant behavior and translated theta frequency selectivity into spiking (resonant cells), while the other 80% acted as low-pass filters with no frequency preference in their discharge (non-resonant cells). Measurement of $I_{NaP}$ and $I_M$ in the same cells revealed that at perithreshold membrane potentials the activation level of $I_M$ was generally low, while that of $I_{NaP}$ was high enough to depolarize the neurons toward spike threshold overcoming the subtle hyperpolarizing effect of $I_M$. This explains the more abundant non-resonant perithreshold behavior. After partial pharmacological block of $I_{NaP}$ or subtracting it by dynamic clamp, it was possible to turn non-resonant behavior into resonant, demonstrating that the difference between both groups is the activation level of $I_{NaP}$. Furthermore, changing the activation range of $I_M$ towards hyperpolarized potentials by dynamic clamp also transformed non-resonant neurons into resonant. We propose that the relative levels of $I_{NaP}$ and $I_M$ control perithreshold behavior of pyramidal neurons. The fact that these two currents are highly modulated by intracellular signaling and neuromodulators...
provides a fast mechanism to tune perithreshold frequency preference and selective firing in CA1 according to cell and network activity.

**Disclosures:** J.A. Vera: None. J. Alcayaga: None. J. Bacigalupo: None. M. Sanhueza: None.

**Poster**

**480. Neural Oscillators and Activity-Dependent Plasticity of Intrinsic Membrane Properties**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program/#Poster#:** 480.09/B51

**Topic:** B.10. Intrinsic Membrane Properties

**Support:** NIH Grant NS064013

**Title:** Cell-autonomous function of NMDA receptors in the development of intrinsic excitability of thalamocortical neurons

**Authors:** *Z.-W. ZHANG, G. HOU, M. PETERSON, H. LIU;
The Jackson Lab., Bar Harbor, ME

**Abstract:** Neurons and synapses undergo extensive changes in functional properties during early life. In newborns glutamatergic synapses contain few or no AMPA-type receptors (AMPARs) and synaptic transmission is primarily mediated by N-methyl-D-aspartate receptors (NMDARs). Synaptic maturation is associated with an increase of AMPARs at the synapses, and in many brain regions, this upregulation of AMPARs requires NMDAR-mediated signaling. Together with synaptic maturation, the intrinsic properties of neurons show dramatic changes during early life. These include negative shifts in resting membrane potential and action potential threshold, a reduction of input resistance, and an acceleration of action potential kinetics. Little is known, however, about the role of NMDARs in the development of intrinsic excitability of neurons. To address this important question, we deleted NMDARs in a fraction of neurons in the ventral posterior medial nucleus (VPm) of the thalamus using a transgenic Cre strain driven by the serotonin transporter (SERT). In SERT-Grin1 mutant mice, 30-60% of VPm neurons lack functional NMDARs while the remaining neurons have normal NMDAR function. We performed patch clamp recording in acute brain slices from VPm neurons with or without NMDARs. Neurons without NMDARs showed a much higher excitability than those with NMDARs. Compared with VPm neurons with NMDARs, those lacking NMDARs required much smaller currents for activation and showed a higher gain. In addition, neurons without NMDARs showed a reduction of HCN channel function, an increase in input resistance, and
smaller soma. There was no difference in the resting membrane potential or action potential threshold between cells with and without NMDARs, suggesting that the general health of VPm neurons was not affected by NMDAR deletion. In contrast to the effects of NMDAR knockout, deletions of AMPARs had no significant effect on intrinsic properties of VPm neurons. Our results suggest that NMDARs are required for the maturation of intrinsic excitability of thalamic neurons.


Poster

480. Neural Oscillators and Activity-Dependent Plasticity of Intrinsic Membrane Properties

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 480.10/B52

Topic: B.10. Intrinsic Membrane Properties

Title: Aloe vera linn (liliacee) increases brain sodium-potassium atpase activity in streptozotocine-induced diabetic female wistar rats

Authors: *A. O. MAHMUD-IMAMFULANI1,2, E. O. ALAYE2, B. V. OWOYELE2;
1Physiol., 2Univ. of Ilorin, Ilorin, Nigeria

Abstract: Background: Diabetes mellitus (DM) may affect the morphology and plasticity of the brain, leading to cognitive, memory, and electrophysiological impairment. Streptozotocine-Induced (STZ-induced) diabetes leads to a sustained up-regulation of facilitatory adenosine A2A receptors in the hippocampus. Brain Sodium-Potassium ATPase (Na+/K+-ATPase) enhances neuroprotection, memory consolidation and diminishes cognitive deficits. Thus, it has been observed that brain Na+/K+-ATPase activities is reduced in diabetic state in male animals. We therefore aim to determine the effects of caffeine (an adenosine receptor antagonist) on brain Na+/K+-ATPase activities in STZ-induced diabetic female rats. Materials and Methods: Four groups of 7 female Wistar rats each, aged 12-13 weeks old and weighing between 150-200g were used for the study. Group 1 were control; group 2 were diabetic untreated; group 3 were diabetic administered 300mg/kg BW Aloe Vera gel orally and group 4 were diabetic administered 300mg/kg BW Aloe vera pulp and gel. DM was induced with 50 mg/kg STZ. At the end of the experiments, rats were anaesthetised, brains were removed, weighed and homogenized in sucrose solution and kept at 40C. Protein determination and assay for the enzyme were carried out within 24 hours of sample collection. Na+/K+-ATPase activities were expressed as µmolPi/mg protein/hour x 10-3. Results: The results showed a significant (P < 0.05) decrease in Na+/K+-
ATPas activities of untreated DM group (250.9 ± 0.26) when compared with control group (415.6 ± 0.26). Also groups 2 and 3 treated with Aloe vera gel and Aloe vera pulp & gel showed significant (P < 0.05) increase in Na+/K+ -ATPase activities (466.8± 0.48 and 473.5± 0.71 respectively) when compared with diabetic untreated group. Conclusion: In conclusion, the findings from this study shows that Aloe vera linn pulp and gel improves and restores the brain Na+/K+-ATPase activities in diabetic rats. Thus moderate Aloe Vera intake could be beneficial to brain functions in diabetic animals and man.


Poster

481. Oligodendrocytes and Progenitor Biology

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 481.01/B53


Support: JSPS KAKENHI 25461794

Title: Novel systems for isolation and functional analysis of adult NG2 condroitin sulfate proteoglycan-expressing cells

Authors: *N. KIKUCHI(NIHONMATSU), X. YU, Y. MATSUDA, M. WATANABE, Y. TATEBAYASHI;
Tokyo Metropolitan Inst. of Med. Sci., Tokyo, Japan

Abstract: NG2 condroitin sulfate proteoglycan-expressing cells (NG2 cells) are widely distributed throughout the brain, both in gray matter and white matter, around 5% of all cells in the adult rodents. The cell cycle time of NG2 cells increased from early postnatal to adult by use of the accumulation of BrdU labeling cells and the production of oligodendrocytes is well known to decrease steadily during adulthood, suggesting that adult NG2 cells might play a more important role as the maintenance of physiological conditions rather than the production of oligodendrocytes and further that there are different characters between the adult and developmental NG2 cells. We have recently established a novel method to isolate and expand NG2 cells from adult rat brains (A-NG2 cultures). In the present study, we confirmed our culture systems for isolation and maintenance of A-NG2 cultures in detail. To isolate NG2 cells from other CSF cells, we used the buoyant density by a step gradient with OptiprepTM. A major thick layer at the lower density than microglia and neuronal stem cells was apparent after centrifugation, while a precipitation was found at the bottom of the tube (P fraction). After a
major layer was expanded on dishes more than for 30min, the supernatants including non-adherent cells and the debris were removed from dishes (sup-wash fraction). Western blot analysis reveled that sup-wash fraction and P fraction contained the debris or cells of microglia and myelin. A-NG2 cultures were effectively grown and maintained in basic fibroblast growth factor (FGF2) instead of platelet-derived growth factor (PDGF), suggesting that FGF2 was crucial for proliferation of adult NG2 cells, whereas many previous reports have shown that PDGF was required for proliferation of developmental NG2 cells in vitro and in vivo. The morphology of the A-NG2 cultures containing FGF2 was consistent with those of adult NG2 cells in vivo. B27 minus antioxidants (B27-AO) caused apoptosis and inhibited proliferation. Furthermore, we found the new functions associated with neurodegenerative disorder on adult NG2 cells by using our culture system. Taken together, our data is highly suggestive that A-NG2 cultures are novel systems for investigating the physiological mechanisms of NG2 cells in the adult brain.


Poster

481. Oligodendrocytes and Progenitor Biology

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 481.02/B54


Support: State Key Program of National Natural Science of China(Grant No. 81030061)

National Natural Science Foundation of China (Grant No. 81273490)

Foundation for Innovative Research Groups of the National Natural Science Foundation of China (Grant No. 81221003)

Title: IL-1β impedes oligodendrocyte progenitor cell migration after chronic cerebral hypoperfusion

Authors: *L. JIANG¹, J. ZHANG², Z. CHEN¹, W. HU¹;
¹Dept. of Pharmacology, Col. of Pharmaceut. Sci., Zhejiang Univ., Zhejiang, China; ²Zhejiang Univ. Sch. of Med., Sir Run Run Shaw Hosp., Hangzhou, China

Abstract: Subcortical ischemic vascular dementia (SIVD) induced by chronic hypoperfusion is a common subtype of vascular dementia, with characteristics of white matter damage and
cognitive behavior impairment, yet the detailed pathogenic mechanism is still unclear. Our previous and others work imply that glial activation participates in the development of SIVD. IL-1β is one of the major proinflammatory cytokines secreted by glia, however, its role in SIVD is not appreciated. We found that IL-1β elevated from 1d to 7d and returned to baseline at 14d in corpus callosum after right unilateral common carotid arteries occlusion (rUCCA0).

Administration of IL-1 receptor antagonist (IL-1Ra), from 1d to 7d (1 µg, i.c.v.), rescued the downregulation of MBP caused by hypoperfusion on 34d. Electron microscopy showed that IL-1Ra treatment increased the number of myelinated axons after rUCCA0. In IL-1 receptor knockout (IL-1R KO) mice, there is no difference between rUCCA0 group and sham group both in MBP expression and myelin numbers, while the percentage of axons with thin myelin increased after hypoperfusion. We also showed that IL-1 Ra reversed the decrease of NG2+ oligodendrocyte progenitor cells (OPCs) number in corpus callosum at 8d after hypoperfusion, however IL-1R KO mice did not display obvious loss of OPCs after hypoperfusion. Besides, by using Brdu labeling we confirmed that IL-1β did not affect the proliferation of OPCs in SVZ. However, we found that IL-1 Ra and IL-1R KO increased the number of Brdu+ NG2+ cells in corpus callosum after hypoperfusion, which suggests that IL-1β inhibits OPCs recruitment from SVZ to corpus callosum to inhibit remyelination. To further verify that, the proliferating newborn cells in SVZ are traced by retrovirus injection, and we found that IL-1Ra treatment elevated newborn cell number in corpus callosum, which can be reversed by co-treatment with IL-1β. In addition, IL-1R KO mice also exhibited the upregulation of newborn cell number in corpus callosum after rUCCA0. Moreover, IL-1 Ra administration significantly rescued the decrease of the discrimination index in object cognition test, the increase of escape latency during the acquisition trials and the reduction of time spent in the target quadrant in Morris water test at 28d after rUCCA0, while IL-1R KO animals did not exhibit any cognitive impairment after hypoperfusion, which suggests that IL-1β impaired learning and memory ability caused by hypoperfusion. In conclusion, the upregulation of IL-1β at early stage after rUCCA0 results in poor remyelination after chronic hypoperfusion through blocking OPCs migration. So, IL-1β may serve as a potential therapeutic target for SIVD.

Disclosures: L. Jiang: None. J. Zhang: None. Z. Chen: None. W. Hu: None.

Poster

481. Oligodendrocytes and Progenitor Biology

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 481.03/B55

Support: Mathers Foundation

Yerkes Base Grant (NIH Office of Research Infrastructure Programs/OD P51OD11132)

Title: Molecular compartmentation of cerebral white matter revealed with Perls iron stain

Authors: *N. M. SINGLETARY¹², J. M. DOOYEMA³, D. A. GUTMAN⁴, T. M. PREUSS¹;

Abstract: Previous studies of iron-stained histological sections have characterized the distribution of iron in cerebral white matter as “patchy” (e.g., Connor et al., 1990, J Neurosci Res 27:595-611; Todorich et al., 2009, Glia 57:467-478). However, the three-dimensional distribution of iron staining has never been determined. To examine this distribution, we made serial reconstructions through human frontal and temporal cortex sections stained with the Perls DAB method, which preferentially stains for ferric iron. We stained 25 micron-thick formalin-fixed, paraffin-embedded sections from the frontal lobes of 16 humans, 14 chimpanzees, and 8 rhesus macaques for Perls DAB using identical incubation conditions and times. All tissues were collected at autopsy or necropsy in accordance with approved IRB and IACUC protocols. All three species showed the expected patchy patterns, but staining density was much greater in humans than in chimpanzees or macaques. Therefore, we carried out our serial reconstruction in humans. We mounted 19-30 consecutive 25-micron thick tissue sections from 3 separate paraffin blocks of temporal lobe cortex and 2 blocks of frontal lobe cortex onto slides and stained them for Perls DAB. After scanning the tissue sections into digital images, we registered the images with the bUnwarpJ function in Fiji (ImageJ), using blood vessels as landmarks. We normalized the contrast of the tissue sections before creating a three-dimensional reconstruction of the portion of the block using Fiji’s Volume Viewer plugin. Examination of three-dimensional serial reconstructions reveals that Perls stain-rich and stain-poor territories form elongated and interdigitating “channels” through white matter on the order of 100 microns in cross-sectional diameter. Given the limited size of the reconstructions, we cannot state the exact length of the channels, but some cover the full 750-micron depth of the most complete reconstructions. The significance of these channels is unclear, but there are several possibilities. One is that these channels represent different fiber contingents within the white matter. Alternatively--or complementarily--the compartments represent different types of oligodendrocytes, one enriched in iron (or ferric iron) and one not.


Poster

481. Oligodendrocytes and Progenitor Biology
**Title:** Olig2+ progenitors and gnas tumor suppressor in shh-medulloblastoma

**Authors:** *H. XUELIAN, L. ZHANG, R. LU; Dept. of Pediatrics Cancer & Blood Dis. Inst. Cincinnati Children', Brain Tumor Ctr., Cincinnati, OH

**Abstract:** Medulloblastoma, the most common malignant childhood brain tumor, exhibits distinct molecular subtypes and cellular origins. Genetic alterations driving medulloblastoma initiation and progression remain poorly understood. Recently we identify GNAS, encoding the G-protein Gsα, as a potent tumor suppressor gene that defines a subset of aggressive Sonic Hedgehog (Shh)-driven human medulloblastomas. Ablation of the single Gnas gene in anatomically-distinct progenitors is sufficient to induce Shh-associated medulloblastomas, which recapitulate their human counterparts. Gsα is highly enriched at the primary cilium of granule neuron precursors and suppresses Shh-signaling by regulating both the cAMP-dependent pathway and ciliary trafficking of Hedgehog pathway components. Elevation of a Gsα effector, cAMP, effectively inhibits tumor cell proliferation and progression in Gnas mutants. Furthermore, we identify a population of Olig2-expressing progenitor cells are the major source of tumor-propagating cells. Deletion of Olig2 leads to a delay of tumor progression. Eliminating the mitotic Olig2-expression inhibits tumor initiation. Thus, our present studies identify a previously unrecognized tumor suppressor function for Gsα that acts as a molecular link across Shh-group medulloblastomas of disparate cellular and anatomical origins, and reveal that Olig2-expressing progenitors are the critical cell origin of G-protein-mediated SHH subgroup medulloblastoma, illuminating potential therapeutic avenues for SHH medulloblastoma by targeting G-protein and Olig2-expressing glial progenitors.

**Disclosures:** H. Xuelian: None. L. Zhang: None. R. Lu: None.
Title: Fkbp5/FKBP51 mediates excitotoxin-induced oligodendrocyte damage

Authors: *S.-H. LIN1,2, S.-Y. CHUANG1,2, Y.-L. GAN1,2, Y.-H. LEE1,2;
1Physiol., 2Natl. Yang-Ming Univ., Taipei, Taiwan

Abstract: White matter damage, as defined by the axon-oligodendrocyte (OLG) damage and demyelination, is a major cause of functional disability and cognitive deficit in various brain injuries and neurodegenerative diseases such as ischemic stroke, Alzheimer’s disease and schizophrenia. Our previous study has demonstrated the glucocorticoid receptor (GR)-mediated protection of OLGs against excitotoxicity via upregulating erythropoietin (Epo). Here we further revealed that a GR cochaperon FK-506 binding protein 51 (FKBP51, encoded by Fkbp5 gene), a GR target gene that mediates a negative feedback loop to maintain the homeostasis of GR activity and stress response, is elevated by excitotoxic AMPA stimulation in primary rat OLGs as well as excitotoxic NMDA stimulation in primary rat cortical neurons. The elevations of Fkbp5 mRNA and FKBP51 were accompanied with a reduction of myelin basic protein (MBP) and PLP expression in AMPA-treated OLGs, and these effects were reversed by a pretreatment with AMPA receptor antagonist CNQX. Furthermore, we found that knockdown of Fkbp5 expression with specific siRNA can attenuate the AMPA-induced MBP reduction and enhance Epo expression in OLGs. These results suggest that elevation of Fkbp5/FKBP51 may contribute to the excitotoxicity-induced demyelination process that leads to white matter degeneration.

Disclosures: S. Lin: None. S. Chuang: None. Y. Gan: None. Y. Lee: None.
Abstract: Temporal precision required in neural system processing can range from the sub-millisecond in sound localization and echolocation, to milliseconds and hundreds of milliseconds in perceptual and motor processing tasks. At a level of signal conduction through axons between individual neurons, the arrival time of action potentials from different neurons requires millisecond precision. It has been proposed that to achieve such precise timing, adaptive regulation of these temporal delays is required. Dynamic modulation of myelination potentially provides one mechanism to control impulse arrival times. However, the focus here is on general consequences of having adaptive time delays, rather than on the precise biological mechanism. We divide “delay plasticity” models into two groups based on the feedback mechanism they utilize. The first is activity-dependent delay plasticity (ADDP), in which the learning rule modifies the time delays based on the locally observed activity level; the second is temporal-mismatch delay plasticity (TMDP). These two learning paradigms were implemented using a framework of delay coupled non-linear oscillators and spiking neural networks with adaptive delays (where TMDP becomes spike-timing-dependent delay plasticity (STDDP)). In both these models we compare and contrast the roles played by synaptic and delay plasticity. We show how a simple local ADDP rule stabilizes a system of coupled oscillators and extend this finding to the case of spiking neural networks. The STDDP is studied in the context of temporal sequence learning. Traditionally, sequence learning has been modeled in terms of specialized neural network architectures implementing tapped delay lines, temporal sequence selection through the mechanisms of spike-timing-dependent plasticity (STDP), or through synaptic triads, and other mechanisms utilizing synaptic plasticity. In this work we introduce a simple feed-forward neural network for sequence learning that utilizes both STDP and STDDP as an effective model for comparing the two plasticity modes. Both detect a mismatch locally at the synapse, but the latter additionally requires retrograde transport, which is inherently slower. We argue that implementing both plasticity mechanisms is needed to provide an optimal trade-off between the speed on one hand, and the efficiency and precision on the other. Our results indicate that the delay plasticity, such as myelin plasticity, is a highly efficient way to make a system stable and learn temporally precise tasks efficiently.

Disclosures: S. Pajevic: None. P.J. Basser: None. R.D. Fields: None.

Poster

481. Oligodendrocytes and Progenitor Biology

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 481.07/B59

Support: DFG SPP 1757
DFG SFB 1089

Title: Role of A-type $K^+$ currents in synaptic integration and calcium signaling in NG2 glial cells

Authors: *W. SUN, D. DIETRICH;
Univ. Clin. Bonn, Bonn, Germany

Abstract: It is well established that NG2 expressing oligodendrocyte precursor cells receive direct synaptic inputs from neurons. The functional role of neuron-NG2 synaptic transmission still remains unclear. The morphological and physiological properties of neuron-NG2 synapses are similar to classic neuron-neuron synapses. However, the output of synaptic integration in NG2 cells differs from neurons due to the absence of action potentials. Thus, integration of excitatory post synaptic potentials (EPSPs) in NG2 cells is graded and does not follow “all-or-none” behavior. In this study we demonstrated that especially A-type $K^+$ currents play a pivotal role for integration by curtailing EPSPs while the amplitude of EPSPs was barely altered due to compensatory recruitment of fast voltage-activated $Na^+$ currents. It has been speculated that neuronal activity could activate postsynaptic signaling pathways which are important for the proliferation and/or differentiation of NG2 cells. We discovered that $Ca^{2+}$ signaling could be directly triggered by mock synaptic potentials through activation of voltage-gated $Ca^{2+}$ channels. These somatic $Ca^{2+}$ transients also back-propagated to distal dendrites. Interestingly, blockade of A-type $K^+$ currents significantly increased the fraction of detectable $Ca^{2+}$ transients and also increased the amplitude of $Ca^{2+}$ transients. Therefore, our results suggest that A-type $K^+$ currents are important for gating $Ca^{2+}$ entry into NG2 cells. Finally, by combining 2-photon glutamate uncaging and 2-photon $Ca^{2+}$ imaging, we revealed that in the absence of A-type $K^+$ currents, NG2 cells could discriminate additional synaptic inputs during an ongoing global postsynaptic depolarization. This discrimination was represented by local dendritic calcium signals at individual dendritic branches, and the somatic electrical response was hardly altered. Therefore it appears that local calcium signaling, gated by dendritic A-type $K^+$ channels, serves as a localization indicator for additional synaptic inputs in NG2 cells. In summary, our study suggests a pivotal role of A-type $K^+$ channels in gating synaptic integration and calcium signaling in NG2 cells, which is clearly different from classical synaptic integration known from neurons.

Disclosures: W. Sun: None. D. Dietrich: None.

Poster
481. Oligodendrocytes and Progenitor Biology

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM
Program#/Poster#: 481.08/B60

**Topic:** B.11. Glial Mechanisms

**Support:**
- NINDS K12 NS079414 (CME)
- NINDS R25 NS070682 (CME)
- Fred Lovejoy Resident Research Award (CME)
- Baby Alex Foundation Grant (CME & PAR)
- NIH EY024481 (PAR)
- National Multiple Sclerosis Foundation Pilot Grant (PAR)
- NIH P50 HD 018655

**Title:** Probing intracellular zinc status in developing and mature oligodendrocytes

**Authors:** *C. M. ELITT*1,2, J. WANG2, A. BACH2, C. J. FAHRNI3, P. A. ROSENBERG1,2;  
1Neurol., Boston Children's Hosp., Boston, MA; 2F.M. Kirby Neurobio. Ctr., Boston Children's Hosp. and Harvard Med. Sch., Boston, MA; 3Sch. of Chem. and Biochem. and Petit Inst. for Bioengineering and Biosci., Georgia Inst. of Technol., Atlanta, GA

**Abstract:** Zinc homeostasis is important for oligodendrocyte (OL) survival but remains poorly understood. Prior studies have demonstrated that increases in cytosolic mobile or free ionic zinc are essential for excitotoxic and nitrative injuries to mature OLs (Mato et al., 2013, Zhang et al., 2006) but few studies have been pursued in developing OLs. We hypothesized that zinc handling might be developmentally regulated in OLs. Mixed glia cultures were isolated from postnatal day 2 (P2) rat forebrains and grown for 10-17 days. Developing OLs were separated from microglia and astrocytes using selective detachment and then plated onto coverslips. Cells were maintained in the presence of platelet-derived growth factor (PDGF) and basic fibroblast growth factor (FGF) for 8 days prior to imaging. To produce mature OLs, PDGF and FGF were replaced by triiodothyronine (T3) and ciliary neurotrophic factor (CNTF). Zinc imaging was performed using Chromis-1, a new Zn(II)-selective ratiometric fluorescent probe that has been optimized for two-photon excitation fluorescence microscopy (TPEM). Baseline fluorescence measurements were collected in developing and mature OLs preloaded with Chromis-1 and then cells were treated with either exogenous zinc (1 nM) combined with sodium pyrithione (2 nM) or the transition metal ion chelator, N,N,N',N'-tetrakis(2-pyridylmethyl)ethane-1,2-diamine (TPEN, 10 µM). For each condition, we measured the two-photon excited fluorescence intensity through two bandpass filters (425-462 nm and 478-540 nm, respectively), and calculated the corresponding intensity ratio image for the two channels. The Zn(II) saturation of Chromis-1 was significantly increased in developing vs mature OLs as judged from the different intensity ratios of 1.25 ± 0.07 vs. 0.65 ± 0.12, respectively (n=5, P<0.005; total cells imaged included 158 developing OLs...
and 169 mature OLs). Furthermore, the intensity ratio was decreased by chelation with 10 µM TPEN and increased by application of exogenous zinc at concentrations as low as 1 nM. Our data suggest that buffered cytosolic Zn(II) is downregulated as oligodendrocytes mature. Chromis-1 represents an important new tool for investigating zinc homeostasis in oligodendrocytes and other cells under physiologic and pathophysiologic conditions. Future studies will investigate the role of changing intracellular zinc levels in oligodendrocyte development and in response to injury.

**Disclosures:** C.M. Elitt: None. J. Wang: None. A. Bach: None. C.J. Fahrni: None. P.A. Rosenberg: None.

**Poster**

481. Oligodendrocytes and Progenitor Biology

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 481.09/B61

**Topic:** B.11. Glial Mechanisms

**Support:** NIDCD (R01 DC03157)

**Title:** Oligodendrocytes beyond the precursor stage generate Na_1.2-driven action potentials into early adulthood

**Authors:** *E. BERRET*, K. JUN HEE;
Physiol., UTHSCSA, San Antonio, TX

**Abstract:** Oligodendrocyte maturation and axon-glial communication are required for proper myelination in the developing brain. Although axon myelination continues until early adulthood, the physiological characteristics of oligodendrocyte lineage cells remain largely understudied in mature brains. Oligodendroglia excitability is a potentially important mechanism for promoting axon myelination, but the necessity of Na⁺ channel and electrical excitability of oligodendroglia in relation to their maturation to myelinating stage is controversial. We investigated the spiking properties of oligodendrocytes in their pre-myelinating stage (pre-OLs; CNPase-positive, NG2-negative) and compared them with spiking in oligodendrocyte precursor cells (OPCs, NG2-positive). We found two distinct classes of pre-OLs: one had typical passive membrane properties, while the other expressed substantial voltage-activated Na⁺ currents and generated action potentials in response to depolarizing current injections. Electrophysiological, pharmacological, and immunostaining data demonstrate that spiking in excitable pre-OLs was driven by Na_1.2 channels. Of particular interest is that excitable pre-OLs were detected
throughout postnatal development up to postnatal day 62. Thus, Na\textsubscript{v}1.2-driven spiking is not limited to OPCs nor to a transient stage of brain development. Rather, spiking behavior of immature oligodendrocytes persists into the young adult brain. These results indicate that spiking in oligodendrocyte lineage cells is required for their normal differentiation and maturation.

Disclosures: E. Berret: None. K. Jun Hee: None.

Poster

481. Oligodendrocytes and Progenitor Biology

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 481.10/B62


Support: 5T32GM007839-32

5T32GM007839-33

NIH Grant NS34939

Title: The integrated stress response in perinatal diffuse white matter injury

Authors: *B. CLAYTON, B. POPKO;

Univ. of Chicago, Chicago, IL

Abstract: Perinatal diffuse white matter injury (DWMI) is a disorder associated with premature birth, which is increasing in prevalence and leads to cognitive and behavioral deficits in 40-50% of preterm infants. PWMI is caused by hypoxic, ischemic, and inflammatory insults that damage oligodendrocyte precursor cells (OPCs) leading to reduced myelination. We are currently exploring the integrated stress response (ISR) in PWMI. The ISR is a conserved cellular stress response initiated by a variety of cellular stresses, which lead to phosphorylation of eIF2\textalpha through activation of one of four stress sensing eIF2\textalpha kinases. Phosphorylation of eIF2\textalpha inhibits global protein translation while selectively increasing the translation of the transcription factor ATF4. ATF4 induces the expression of cytoprotective proteins, including anti-oxidant enzymes. Importantly activation of the ISR is initially protective, while prolonged activation of the ISR in the face of unresolved stress can lead to apoptosis via CHOP. In non-neuronal cells hypoxia and ischemia activate the ISR, which provides protection from these insults. Here, we present data showing that the ISR is activated in OPCs following oxygen-glucose deprivation (OGD), an in vitro model of hypoxia and ischemia. Moreover, inhibition of the ISR sensitizes OPCs to OGD and enhancement of the ISR provides increased protection. Additionally, we present data
demonstrating that genetic manipulation of the ISR affects myelin outcome in mouse models of perinatal diffuse white matter injury. This study addresses the role of the ISR in survival of OPCs during hypoxia and ischemia and myelination in perinatal DWMI.

**Disclosures:** B. Clayton: None. B. Popko: None.

**Poster**

481. Oligodendrocytes and Progenitor Biology

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 481.11/B63

**Topic:** B.11. Glial Mechanisms

**Support:** KAKENHI 25460399

**Title:** Experimental ablation of NG2-expressing glial progenitor cells induced neuronal cell death by pro-inflammatory pathway

**Authors:** *M. NAKANO*¹,³, Y. TAMURA¹,², A. EGUCHI¹,², M. YAMATO¹,², S. KUME¹,², Y. KATAOKA¹,²,³,

¹Multi-Modal Microstructure Analysis Unit, ¹RIKEN Ctr. for Life Sci. Technologies, Kobe / Hyogo, Japan; ³Osaka City Univ. Grad. Sch. of Med., Osaka, Japan

**Abstract:** Glial progenitor cells expressing chondroitin sulfate proteoglycan 4 (NG2) are termed as NG2 glial cells (or oligodendrocyte precursor cells). NG2 glial cells comprise the majority of proliferative cells in the adult central nervous system (CNS). They also can migrate, proliferate, differentiate and modulate the neuronal network in response to the alteration of surrounding environments, such as acute CNS injury or neuronal activation. Although NG2 glial cells have a compensating function to keep the constant cell density in the tissue, the ability gradually declines with aging. These findings support the hypothesis that NG2 glial cells maintain the homeostasis in adult CNS, and that the dysfunction of them leads to impairment of neuronal function and neurodegeneration such as Alzheimer's disease. To test this hypothesis, we have generated transgenic rats expressing herpes simplex virus thymidine kinase (HSVtk) under the control of the promoter for NG2 (NG2-HSVtk Tg rats). HSVtk is a suicide gene that converts ganciclovir (GCV), a synthetic analog of nucleoside into a toxic triphosphate form, which can be incorporated into genome and terminate DNA synthesis. Thus, proliferative cells expressing HSVtk are sensitive to GCV compared with non-dividing cells. Therefore, this strategy allows
selective ablation of NG2 glial cells in NG2-HSVtk Tg rats. By continuous administration of GCV into the lateral ventricle, we succeeded in rapid degeneration of NG2 glial cells in the surrounding area of the ventricle including the hippocampus. Following ablation of NG2 glial cells, hippocampal neurons were gradually degenerated. Moreover, expressions of interleukin (IL)-1β, IL-6 and TNFα were dramatically up-regulated following ablation of NG2 glial cells, and then a large number of activated microglial cells appeared. The expression of Bcl-2, anti-apoptotic gene, was also down-regulated after the ablation of NG2 glial cells. These data indicated that hippocampal neurons were vulnerable to neuroinflammation in the NG2 glial cell-ablated brain. To further investigate the mechanism of neuronal defect, we inhibited the IL-1β pro-inflammatory pathway by the co-administration of IL-1 receptor antagonist (IL-1ra), which is an endogenous competitive antagonist for IL-1 receptors. The treatment of IL1ra attenuated the neuronal cell death induced by ablation of NG2 glial cells. These results suggested that the ablation of NG2 glial cells leads to neuronal cell death through the pro-inflammatory IL-1β pathway.


Poster

481. Oligodendrocytes and Progenitor Biology

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 481.12/B64


Support: German Research Foundation STE 552/5

German Research Foundation SFB 645

Title: Gray matter NG2 cells release exosomes that contain non-alpha GFAP isoforms and NG2

Authors: *R. JABS¹, J. WALTER², M. VAN STRIEN³, E. M. HOL³, C. STEINHÄUSER¹, K. GLEBOV²;
¹Inst. of Cell. Neurosciences, Univ. of Bonn, Bonn, Germany; ²Dept. of Neurol., Univ. of Bonn, Bonn, Germany; ³Dept. of Translational Neurosci., Univ. Med. Ctr. Utrecht, Utrecht, Netherlands

Abstract: NG2 cells are the only non-neuronal cells in the CNS that receive synaptic input from pre-synaptic neurons. Post-synaptic signals are sensed by a variety of voltage- and ligand-
activated ion channels. However, the physiological relevance of this synaptic signaling is enigmatic. It is known that NG2 cells express multiple Ca2+-signaling pathways. In other glial cell types, namely microglial cells and oligodendrocytes, neurotransmitters activate Ca2+-dependent signaling pathways that regulate the release of glial exosomes. Finally, it has been shown that cleaved NG2 is able to modulate neuronal networks in an activity-dependent manner. This experimental evidence raises the question whether NG2 cells also release exosomes. In this study we demonstrate first Simvastatin-evoked release of exosomes from Oli-neu cells, a well-established culture model of NG2 cells. Oli-neu derived exosomes contain the marker proteins Alix, insulin degrading enzyme (IDE), and flotillin-1. In addition, we also demonstrate the presence of glial fibrillary acidic protein (GFAP) and NG2 itself as cargo of the exosomes. PCR analysis revealed different non-alpha GFAP isoforms in Oli-neu cells. Immunoblotting of exosome preparations indicated at the protein level non-alpha GFAP isoforms being part of exosome cargo. Fluorescence microscopy revealed a close spatial association of flotillin-1, IDE and GFAP in the soma and also in the typical fine processes of Oli-neu cells. Next we analyzed authentic NG2 cells in acute brain slices and combined electrophysiological recordings with single cell RT-PCR. These experiments detected mRNA for GFAP isoforms in NG2 cells and also confirmed the absence of the GFAP alpha isoform in situ. Taken together, our results indicate exosomal release of non-alpha GFAP isoforms and NG2 from NG2 cells. This pathway might allow efficient feedback signaling from NG2 cells in response to synaptic input from neuronal pre-synapses.


Poster

481. Oligodendrocytes and Progenitor Biology

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 481.13/B65


Support: NIH NRSA F31NS081905

Title: Jam2 inhibits somatodendritic myelination of neurons by oligodendroglia

Authors: *S. REDMOND*1, Y. ESHED-EISENBACH2, F. MEI1, L. OSSO1, Y.-A. SHEN1, S. Y. C. CHONG1, E. PELES2, J. R. CHAN1;

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**Abstract:** It has long been known that myelination occurs solely around neuronal axons. It has previously been shown that oligodendrocytes, the myelin-forming cells of the central nervous system (CNS), are able to myelinate nanofiber substrates above a certain threshold diameter, in the absence of any molecular cues. Interestingly, the gray matter of the CNS contains super-threshold diameter somata and dendrites, which are never myelinated. Utilizing a purified spinal cord neuron myelinating coculture system, we show that the somatodendritic compartment is not myelinated *in vitro* when neuro-glial signaling is intact. When dynamic signaling between neurons and oligodendrocytes is interrupted by chemical fixation, oligodendrocytes no longer avoid wrapping myelin membranes around neuronal somata and dendrites *in vitro*. We have utilized the spinal cord neuron myelinating coculture system to screen for novel signaling molecules that modulate oligodendrocyte myelination of somata and dendrites. We have identified Junction Adhesion Molecule 2 as necessary and sufficient to inhibit oligodendrocyte myelination. Taken together, this work furthers the understanding of the nature of attractive, permissive and/or inhibitory signals that shape CNS myelination.

**Disclosures:** S. Redmond: None. Y. Eshed-Eisenbach: None. F. Mei: None. L. Osso: None. Y. Shen: None. S.Y.C. Chong: None. E. Peles: None. J.R. Chan: None.

**Poster**

**481. Oligodendrocytes and Progenitor Biology**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program/Poster#:** 481.14/B66

**Topic:** B.11. Glial Mechanisms

**Support:** DFG-EXC 307

**Title:** Response of white matter oligodendrocyte precursor cells to different patterns of neuronal activity *in situ* and *in vivo*

**Authors:** *B. Nagy*¹, A. Hovhannisyan¹², R. Barzan¹, M. Kukley¹; ¹Werner Reichardt Ctr. For Integrative Neuroscien, Tuebingen, Germany; ²Univ. of California, Santa Cruz, CA

**Abstract:** Oligodendrocyte precursor cells (OPCs) are widely distributed throughout the central nervous system. They generate myelinating oligodendrocytes (OLs) during postnatal brain development. Also in adult age OPCs produce new OLs which restore myelin-sheaths in conditions resulting in loss of myelin. OPCs express ionotropic receptors for glutamate, and are involved in synaptic signaling with neurons. Those receptors are located at the postsynaptic sites...
of neuron - OPC synapses. It has been suggested that the glutamatergic synaptic input plays a role in the OPCs' proliferation or differentiation, but the results are ambiguous. In freely behaving mice many neurons fire bursts or short trains of action potentials, but the functional properties of axon - OPC synapses during repetitive neuronal activity have not been investigated. Studies on neuron - OPC synapses generally focused on the responses of OPCs to single or double pulse stimulation. Here we investigated the synaptic response of OPCs to different train stimulations of callosal axons \textit{in situ}. We also studied the effects of different stimulation paradigms applied to callosal axons in freely behaving mice, on OPC proliferation and differentiation \textit{in vivo}. We found that the response of an OPC to repetitive stimulation of callosal axons in brain slices consisted of two components: phasic and asynchronous. The phasic response was time-locked to the stimulation pulse while the asynchronous responses continued for up to several hundreds of milliseconds after the stimulation ceased. Stimulation of callosal axons with 20 pulses at 25 or 100 Hz triggered substantial potentiation of the phasic response; while stimulation with 20 pulses at 300 Hz the potentiation was negligible. The rate of asynchronous responses also depended on the stimulation frequency: if the number of pulses was kept constant, 300 Hz trains triggered the least, while 25 and 100 Hz trains triggered the most asynchronous responses. If the stimulation frequency was kept constant, the asynchronous component was more pronounced after the longer than after the shorter trains. Our data demonstrate that callosal OPCs in brain slices use synapses with axons to detect different pattern of axonal activity. To test whether OPCs respond in a distinct way to different patterns of stimulation also \textit{in vivo} we repeatedly stimulated the corpus callosum of freely moving mice with 20 pulses at 25 or 300 Hz. We found that the 25 Hz stimulation significantly increased both the proliferation of OPCs and their differentiation into OLs, whereas 300 Hz stimulation didn't. Thus, proliferation and differentiation of OPCs \textit{in vivo} may depend on the pattern of neuronal activity.

**Disclosures:** B. Nagy: None. A. Hovhannisyan: None. R. Barzan: None. M. Kukley: None.

**Poster**

481. Oligodendrocytes and Progenitor Biology

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program/#Poster#:** 481.15/B67

**Topic:** B.11. Glial Mechanisms

**Support:** DFG-EXC307
Title: Functional role of AMPA receptors during differentiation of oligodendrocyte precursor cells in mouse corpus callosum

Authors: *T.-J. CHEN¹, A. GALL¹, I. EHRLICH¹², M. KUKLEY¹;
¹Werner Reichardt Ctr. for Integrative Neuroscien, Tübingen, Germany; ²Hertie Inst. for Clin. Brain Res., Tübingen, Germany

Abstract: Oligodendrocyte precursor cells (OPCs), the progenitors of oligodendrocytes (OLs), are widespread in the grey and white matter regions of the central nervous system. OPCs in grey and white matter receive glutamatergic synaptic input from neurons. Transition of OPCs to the premyelinating stage is accompanied by the rapid removal of glutamatergic synaptic input, including downregulation of AMPA receptors (AMPARs). Furthermore, treatment of cultured cerebellar slices with AMPA causes a decrease in the percentage of O1+ cells, as well as in the level of CNP mRNA, pointing to the fact that AMPA application reduces differentiation of oligodendrocyte lineage cells. Based on these findings, we hypothesized that alteration of AMPAR-mediated signaling in OPCs may influence their differentiation into OLs. To test this hypothesis, we first verified the composition of AMPARs in callosal OPCs of NG2DsRed mice at postnatal day 13-17 (P13-17) using electrophysiology. We found that AMPAR-mediated axon-glia currents displayed linear current-voltage (I-V) curve indicating that OPCs contain the GluA2 subunit. Next, we used a viral approach to modify the properties of the GluA2 subunit in different ways specifically in OPCs. Each modifying construct was tagged with green fluorescent protein (GFP) and virus was stereotactically injected into the corpus callosum of mice at P10-12. Animals of the control group received injection of a virus expressing GFP alone. Properties of infected cells were assessed 3-5 days post-injection. To test specificity, we performed immunohistochemistry on brain slices using NG2 and CC1 antibody to label cells of the oligodendroglia lineage. We found that more than 90% of GFP+ cells were NG2+, CC1+, or NG2+CC1+ indicating that the virus specifically targeted cells of the oligodendroglia lineage. To verify the success of GluA2 subunit modification, we recorded the I-V curve of AMPAR-mediated currents in OPCs. We found that in OPCs transfected with a modifying construct, the I-V curve changed from linear to inwardly rectifying indicating that AMPARs with modified properties substituted the endogenous receptors in OPCs in vivo. Finally, we studied the effect of AMPAR modification in OPCs on the differentiation of OPCs into mature OLs in vivo. We found that this resulted in a reduced percentage of CC1+GFP+ cells within the total population of GFP+ cells, indicating a decrease in differentiation of OPCs into mature OLs. Together, our data suggest that during development glutamatergic axon-OPC signaling through AMPARs containing the GluA2 subunit is a key factor for OPCs differentiation in mouse corpus callosum.

Disclosures: T. Chen: None. A. Gall: None. I. Ehrlich: None. M. Kukley: None.

Poster

481. Oligodendrocytes and Progenitor Biology
Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 481.16/B68


Title: Axo-glial interaction preceding CNS myelination is regulated by bidirectional Eph-ephrin signaling

Authors: *L. S. LAURSEN, C. LINNEBERG, M. HARBOE;
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Abstract: In the central nervous system, myelination of axons is required to ensure fast saltatory conduction and for survival of neurons. However, not all axons are myelinated, and the molecular mechanisms involved in guiding the oligodendrocyte processes towards the axons to be myelinated are not well understood. Only a few negative or positive guidance clues that are involved in regulating axo-glial interaction prior to myelination have been identified. One example is laminin, known to be required for early axo-glial interaction, which functions through α6β1 integrin. Here we identify the Eph-ephrin family of guidance receptors as novel regulators of the initial axo-glial interaction, preceding myelination. We demonstrate that so-called forward and reverse signaling, mediated by members of both Eph and ephrin subfamilies, has distinct and opposing effects on processes extension and myelin sheet formation. EphA forward signaling inhibits oligodendrocyte process extension and myelin sheet formation, and blocking of bidirectional signaling through this receptor enhances myelination. Similarly, EphB forward signaling also reduces myelin membrane formation, but in contrast to EphA forward signaling, this occurs in an integrin-dependent manner, which can be reversed by overexpression of a constitutive active β1-integrin. Furthermore, ephrin-B reverse signaling induced by EphA4 or EphB1 enhances myelin sheet formation. Combined, this suggests that the Eph-ephrin receptors are important mediators of bidirectional signaling between axons and oligodendrocytes. It further implies that balancing Eph-ephrin forward and reverse signaling is important in the selection process of axons to be myelinated.

Disclosures: L.S. Laursen: None. C. Linneberg: None. M. Harboe: None.

Poster

481. Oligodendrocytes and Progenitor Biology

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM
**Title:** Organization of oligodendroglial paranodal junctions requires the netrin-1 receptor Unc5b

**Authors:** *O. DE FARIA, JR, J. M. BIN, A. SADIKOT, T. E. KENNEDY; BTRC, Montreal Neurolog. Inst. / McGill Univ., Montreal, QC, Canada

**Abstract:** Netrin-1 and its receptors direct cell and axon migration in the developing CNS. After development is complete, netrin-1 continues to be expressed in the mature CNS and is involved in multiple aspects of adult brain function, including the maintenance of axonal-oligodendroglial paranodal junctions. We have found that the expression of the netrin-1 receptor Unc5b is upregulated in the adult brain, and is enriched at the axonal oligodendroglial paranodal junction of myelinated axons. Our findings indicate that oligodendrocyte-specific deletion of Unc5b in vivo does not alter myelin abundance, but in contrast, in the absence of Unc5b, axoglial paranodes become severely disorganized, with glial loops detached and everted away from the axon, along with mild disruption of compact myelin ultrastructure. As a result, caspr-1 immunoreactivity disperses along the axon, consistent with the aberrant invasion of paranodal components into the adjacent specialized domains. Furthermore, we report that mice lacking Unc5b exhibit a reduced life span. Together, our data reveal a novel contribution for Unc5b to the axoglial apparatus that is required for normal paranode organization.

**Disclosures:** O. De Faria: None. J.M. Bin: None. A. Sadikot: None. T.E. Kennedy: None.

**Poster**

482. Alzheimer's Disease: Risk Factors and Biomarkers

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Title:** Cerebrospinal fluid biomarkers are associated with medial temporal lobe pathology in preclinical AD

**Support:** NIA Grant R01AG037639
**Authors:** *A. P. MERLUZZI*1, D. C. DEAN, III1, C. M. CARLSSON1, S. C. JOHNSON1, O. C. OKONKWO1, J. M. OH1, N. ADLURU1, G. SURYAWANSHI1, H. ZETTERBERG2, K. BLENNOW2, S. ASThana1, H. ZHANG3, A. L. ALEXANDER1, B. B. BENDLIN1;
1Univ. of Wisconsin - Madison, Madison, WI; 2Univ. of Gothenburg, Gothenburg, Sweden; 3Univ. Col. London, London, United Kingdom

**Abstract:** **Background:** Alzheimer’s disease (AD) is characterized by an accumulation of amyloid plaques and neurofibrillary tangles, yet less is known about how these pathologies progress in preclinical AD. Using a novel multi-shell diffusion-weighted imaging technique, we hypothesized that AD pathology measured in cerebrospinal fluid (CSF) in an at-risk population would be associated with altered neural microstructure in the medial temporal lobes (MTL) - regions which are implicated in early AD. **Methods:** CSF was obtained via lumbar puncture from cognitively healthy participants (n=65, age=61.7 ± 6.26) recruited from the Wisconsin Registry for Alzheimer’s Prevention study. It was then analyzed to detect the AD biomarkers neurofilament light protein (NFL), Aβ42, Aβ40, sAPPβ, total tau (tTau), phosphorylated tau (pTau), and the inflammatory markers MCP-1 and YKL-40. Participants also underwent Hybrid Diffusion Imaging (HYDI), and modeling was accomplished with Neurite Orientation Dispersion and Density Imaging (NODDI) to produce measures of neurite density (dendrites and axons), their orientation dispersion, and free water fraction. Linear regression was used to test the relationship between the ratios sAPPβ/Aβ42, tTau/Aβ42, pTau/Aβ42, and Aβ42/Aβ40 on neural microstructure using voxel-wise analysis (SPM12). **Results:** Controlling for age and sex, ratios of sAPPβ/Aβ42, tTau/Aβ42, and pTau/Aβ42 were associated with increased free water fraction in bilateral MTL, and most robustly in the left MTL. In contrast, Aβ42/Aβ40, sAPPβ, tTau, pTau, NFL, Aβ42, and the inflammatory markers were not associated with altered neural microstructure in these regions. **Conclusions:** Left medial temporal lobe structures may be especially susceptible to AD pathology in preclinical populations. In particular, the tTau/Aβ42 and pTau/Aβ42 results suggest a critical role for tau pathology in early AD, but only in conjunction with amyloid deposition. In contrast, the fact that Aβ42/Aβ40 was not associated with an increase in free water fraction suggests that amyloid pathology alone is not sufficient for producing alternations in neural microstructure. Though it is not known what the increase in interstitial water represents at the cellular level, it may be the result of atrophy, axonal loss, inflammation, or myelin alterations. However, the lack of association between NFL and the inflammatory markers on NODDI parameters in these regions argues against axon damage or inflammation. While more studies are needed to determine the precise underlying pathology, these findings further inform the preclinical picture of AD and shed light on the mechanisms of neural injury.

482. Alzheimer's Disease: Risk Factors and Biomarkers

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 482.02/B71

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Pharmacog consortium is funded by EU-FP7 for the Innovative Medicine Initiative (grant n°115009)

Title: Validation of ADFlag®, a diagnostic blood-test for pre-dementia stages of Alzheimer’s disease


Abstract: Alzheimer’s disease (AD) touches 44.4 million people worldwide. AD diagnosis is time-consuming and remains challenging despite progresses in medical imaging and availability of CSF markers. However, identification of pre-dementia stages of the disease remains elusive, rendering stratification of patients enrolled in clinical trial inoperative. This study was designed to confirm the association and to validate the performances of the blood-based ADFlag® test in
the identification of pre-dementia stages of AD. 204 MCI patients enrolled in the Pharmacog cohort were included. A random set of 65 patients, annotated with CSF amyloid-beta peptides (Aβ), Tau and pTau circulating level, hippocampal atrophy and a battery of neuropsychological assessments including MMSE, ADASCog and Boston Naming Test (BNT) were used as a training set. The level of expression of proteins constitutive of the ADFlag® panel was measured in all blood samples, their profile were used to define signatures to differentiate 4 different groups of patients including 3 pre-dementia groups (subjective cognitive impairment (SCI), early mild cognitive impairment (eMCI) and late mild cognitive impairment (lMCI)) within the training set cohort. ADFlag® classification was further validated on a set of 90 patients. In line with the Pharmacog trial design, the ADFlag® panel identified mostly MCI patients, segregated into eMCI (30.0%) and lMCI (51.1%). SCI and putative AD patients were also identified. Consistent with disease progression the age, gender bias, cognitive scales and hippocampal atrophy reflected a proportionally more severe clinical tableau of the disease with the ADFlag® classification. There was no direct association between the ADFlag® classification and Aβ42 level in the CSF, however, a logistic regression analysis showed a significant intercept between Aβ circulating level and ADFlag® classification. This analysis also revealed a significant association between ADFlag® classification and the ADASCog, MMSE and BNT demonstrating a good coherence of this novel diagnostic tool with progressive cognitive loss. Overall, the classification performances of the ADFlag® test were tested against the original diagnostic using a ROC analysis. It showed a predictivity of 72% for SCI patients, 81% for early MCI patients, 82% for late MCI patients and 90% for patients most-likely affected by AD. The ADFlag® panel thus represents one of the first, easy to use blood test, that in combination with standard assessments, can provide physicians with objective information to support stratification of pre-dementia AD patients.


Poster

482. Alzheimer’s Disease: Risk Factors and Biomarkers

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 482.03/B72
Increasing class separation through isometric transformations in Alzheimer's disease diagnostic

Authors: *F. V. CHIRILA, D. L. ALKON;
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Abstract: Many natural phenomena require data classification and increasing separation between two or more data sets. Most of data sets can be linearized through mathematical transformations or they can be linearized locally, i.e. with small variations of related input parameters. Here we propose an algorithm for data classification and increasing separation between linear data sets based on isometric transformations. The practical validation of this method is demonstrated with two Alzheimer's disease diagnostic assays, based on human skin fibroblasts, and lot-to-lot variation of fetal bovine serum. However, the method we propose is general, and has practical application not only for Alzheimer's disease diagnostic but also for other fields such as machine learning, neural networks, data mining, gene expressions, pattern recognition, cognitive psychology, or astronomy. To demonstrate this generality we also validated this algorithm on randomly generated data sets with a 10% additive noise. Different lots of fetal bovine serum had been found to cause changes in the assay outputs. The overlap probability between the Alzheimer's disease (AD) group and age-matched control (AC) group is 1.2x10^-2 for the five lots of fetal bovine serum used in this study. The overlap probability after the algorithm application drops ~7 orders of magnitude to 5.5x10^-9. The percent increase in separability is augmented at least two orders of magnitude for the five lots of fetal bovine serum used in this study.

Disclosures: F.V. Chirila: None. D.L. Alkon: None.

Poster

482. Alzheimer's Disease: Risk Factors and Biomarkers

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 482.04/B73

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NRF 2012R1A2A1A01002881
NRF 2014M3C7A1046047
MRC 2011-0030738
Title: Serum biomarker candidates profiling in Mild Cognitive Impairment and Alzheimer's disease using iTRAQ quantitative proteomics

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Abstract: Development of early diagnosis platform of AD in a simple and non-invasive way using blood is urgently required. Using the isobaric tagging for relative and absolute quantitation (iTRAQ) proteomic approach, we analyzed differentially expressed proteins (DEPs) of mild cognitive impairment (MCI) and AD patients, compared with cognitively normal controls. Serum samples of control, MCI and AD which were selected according to their PiB-PET scores were used for iTRAQ experiment. We have identified 121 DEPs and showed a network model of them based on bioinformatics tools. To check the possibility of these DEPs as potential biomarkers, we estimated the levels of several proteins in serum which were increased in MCI and AD from the iTRAQ result by performing ELISA and some of these proteins showed significant difference among these individuals. Therefore, we suggest that these DEPs from iTRAQ experiment and bioinformatics analysis have possibilities as early detectable biomarkers of MCI and AD.


Poster

482. Alzheimer's Disease: Risk Factors and Biomarkers

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 482.05/B74

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: OP VaVpI - Operační Program Výzkum a vývoj pro inovace

Title: Biomarkers of CSF: Alzheimer's progression tracking
Abstract: Alzheimer’s disease (AD) is a cognitive neurodegenerative disorder characterized by two hallmark pathological lesions in the brain architecture, amyloid plaques and neurofibrillary tangles (NFT). These are caused by the aberrant accumulation of Aβ and tau proteins. Changes in protein levels of these neuropathological hallmark proteins in the cerebrospinal fluid (CSF) are currently used as biomarkers of brain amyloid deposition and neurodegeneration in AD diagnostics. To test for potential novel AD biomarkers and to investigate whether CSF can tell us more about development and progression of AD we have examined CSF of individuals with AD (n=13), MCI (n=11), FTD (n=11) and cognitively normal controls (n=4). Proteins were identified using liquid chromatography tandem mass spectrometry (Orbitrap Fusion) and quantified using a label free method. Mass spectrometry analysis identified a profile of 905 proteins. Among these app. 50 were changes between controls and AD, 20 MCI versus AD. Our preliminary results suggest significant changes among CSF proteins between healthy age-matched individuals and MCI and AD. These potential novel CSF biomarkers reflecting the ongoing neurodegenerative processes may allow us to confirm further and distinguish different stages of AD.


Poster

482. Alzheimer’s Disease: Risk Factors and Biomarkers

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 482.06/B75

Topic: C.02. Alzheimer’s Disease and Other Dementias

Support: JSPS Grant 06035-123332

JSPS and NSF Grant 11033011-000121
**Title:** On-chip detection of tau mutants and 3R:4R tau ratio based on tau’s binding affinity to taxol stabilized microtubules

**Authors:** *S. P. SUBRAMANIYAN¹, M. C. TARHAN², S. L. KARSTEN³, H. FUJITA², H. SHINTAKU¹, H. KOTERA¹, R. YOKOKAWA¹; ¹Kyoto Univ., Kyoto, Japan; ²LIMMS, Inst. of Industrial Science, The Univ. of Tokyo, Tokyo, Japan; ³NeuroInDx Inc., Signal Hill, CA, USA, Signal Hill, CA, CA

**Abstract:** Introduction: Tau protein is a neuronal microtubule (MT) associated protein (MAP) which regulates MT-kinesin dependent fast axonal transportation. Point mutations in tau affecting the binding affinity to MT and splicing site mutation altering 3R:4R tau ratios are biomarkers for early onset of neurodegeneration. Here, we present an on-chip tau detection device comprising of a MT reservoir, channel and an arrowhead shaped collector region, to detect different 3R:4R ratio and major 2N4R mutations (V248L, G272V, P301L, V337M and R406W) using the MT-kinesin system. The detection is based on tau’s isoform and mutant specific binding to taxol stabilized MTs, and their interference with kinesin-MT binding. An increase in tau decoration on MTs resulted in lowering of MT-kinesin binding, TAMRA-labeled stabilized MTs decorated with tau isoforms and mutants were assayed in kinesin coated microfluidic device. MTs binding and gliding in reservoir were concentrated at collector region for fluorescent intensity (FI) measurement. By measuring FI at collector we were able to detect the type of tau decorating the MTs, undecorated MTs were taken as control. We differentiated 1:0 vs 0:1 1:0 vs 1:3 vs 3:1 vs 1:1 and 3:1 vs 0:1 3R:4R tau ratios (p<0.05), and the five mutants were differentiated from wild 2N4R. Experimental method: Tau detection device was fabricated through UV-lithography technique. In brief, MT reservoir, channel and collector were micro fabricated on SU8 photo resist coated on a glass substrate. Wild and mutant tau (1 μM) and different ratio 3R:4R taua (0:1, 1:3, 1:1, 3:1 and 1:0) were incubated with taxol-stabilized TAMRA-labeled MTs (0.5 μM) at 37°C for 30 min off-chip. These MTs in the motility solution were introduced into the device selectively coated with kinesin. The MTs binding and gliding in the reservoir region were concentrated into the collector and FI images were acquired. The images were processed using ImageJ and the FIs were plotted. Results: FI at collector region were significantly (p<0.05) lower for MTs decorated with 0:1 vs 1:0, 1:0 vs 1:3, 3:1 vs 1:1 and 3:1 vs 0:1 3R:4R respectively. Further, the FI at collectors of device assayed with 2N4R tau MTs were significantly lower than mutants. Among mutants, FI for P301L was highest followed by V337M, G272V, V248L and R406W. Conclusion: We found that the kinesin-MT molecular system has a potential to differentiate 3R and 4R taua as well as different 3R:4R ratios, and wild from mutant taua. The degree to which mutations affect the tau binding strength can be determined through this method. The detection system can also be extended to analyze other MAPs and motor proteins.

482. Alzheimer’s Disease: Risk Factors and Biomarkers

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 482.07/B76

Topic: C.02. Alzheimer’s Disease and Other Dementias

Support: NIH Grant AG047537

NIH Grant AG042890

Title: Difference in prevalence of neurogenic markers and regulatory mirna in non-demented with Alzheimer’s neuropathology

Authors: *D. BRILEY*1, B. KRISHNAN2, R. WOLTJER4, G. TAGLIALATELA2, M.-A. MICCI3; 2Dept. of Neurol., 3Dept. of Anesthesiol., 1Univ. of Texas Med. Br., Galveston, TX; 4Dept. of Neurology, Dept. of Pathology, Oregon Hlth. & Sci. Univ., Portland, OR

Abstract: Alzheimer’s Disease (AD) is characterized by dementia, cognitive declines and the presence of amyloid beta plaques and neurofibrillary tangles in the brain. Recent work has identified a group of subjects who remain cognitively intact despite the presence of neuropathological features associated with fully symptomatic AD. These Non-Demented with Alzheimer’s disease Neuropathology (NDAN) subjects represent a unique population in which to study the cellular mechanisms underlying this extraordinary resistance to cognitive decline. Here we tested the hypothesis that preserved cognitive function is correlated with a greater degree of neurogenesis in the hippocampus, a site important for its role in memory and most affected by AD, and determined its potential epigenetic regulation by specific miRNAs. Methods. Paraffin-embedded human tissue representing AD, NDAN, and age-matched healthy control was stained using established immunohistochemical techniques for SOX2, Ki67, doublecortin (DCX), and NeuN. All nuclei were counter-stained using Hoechst. Tiled images of the whole DG were collected to allow for unbiased evaluation of the region, which can vary between subjects, and regions of the DG. Cells positive for each marker were manually counted using ImageJ, and normalized to the total number of cells present in a field (Hoechst-positive objects counted). Fields reflecting different features of the DG (granular layer, sub-granular layer, and hilus) were evaluated separately. Laser Capture Microdissection was used to isolate cells from specific regions of fresh-frozen human hippocampus sections. RNA was isolated and probed using qRT-PCR for miRNA known to regulate neuronal precursor proliferation and maturation. Results. NDAN subjects demonstrate increased expression for markers of neurogenesis, compared with
AD in a site-specific manner. The proportion of DCX and NeuN positive cells were enhanced in the hilus of NDAN compared with AD subjects. Various miRNAs with roles in neurogenesis and neuronal maturation (e.g. miR-25, miR-124, miR-132) were identified as being altered in NDAN compared with control and AD subjects, and particularly miR-25, while increased in AD, was significantly reduced in NDAN. Conclusions. Our data show that the number of progenitor cells in the hippocampus of NDAN subjects is increased as compared to AD subjects, and similar to controls. Furthermore, increased number of progenitor cells in NDAN may be driven through epigenetic modulation as suggested by differences in the level of expression of specific miRNAs. These data strongly suggest that increased neurogenesis correlates with preserved cognitive function in NDAN subjects.


Poster

482. Alzheimer's Disease: Risk Factors and Biomarkers

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 482.08/B77

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: German National Scholarship

Alzheimer Foundation Initiative

Title: Neuro-cognitive mechanisms of simultanagnosia following posterior cortical atrophy

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Abstract: A significant minority of Alzheimer’s disease patients suffer from atypical focal syndromes such as posterior cortical atrophy (PCA). PCA is dominated by initially isolated progressive higher visual and visuo-spatial impairments. One of the major clinical manifestations is simultanagnosia which is the inability to perceive multiple visual objects at the same time. Although widely studied in stroke patients, little is known about the precise neuro-cognitive mechanisms contributing to simultanagnosia in neurodegenerative diseases. This study aimed to (i) specify changes in basic attention parameters underlying symptoms of simultanagnosia, (ii) assess the link to grey and white matter damage, and (iii) integrate those findings into a neuro-
cognitive model of simultanagnosia following PCA. To this end, simultaneous perception of multiple visual objects was tested in 10 PCA patients with verified AD pathology (measured by Pittsburgh compound B positron emission tomography) and 10 healthy aged-matched controls. The critical outcome measure was the percentage of incorrect object identifications. Using whole and partial report of briefly presented letter arrays based on the mathematically formulated ‘Theory of Visual Attention’ we furthermore quantified and compared visual attention parameters across study groups. Patients demonstrated a specific slowing of visual processing speed, while visual short-term memory capacity was preserved. Furthermore, voxel-based morphometry yielded extensive reductions of grey and white matter in parieto-occipital and thalamic brain areas. Among PCA patients, those with slower processing speed showed more severe symptoms of simultanagnosia. Interestingly, the degree of individual atrophy of white but not of grey matter regions was associated with processing speed. Based on these findings, we propose atrophied white matter commonly observed in PCA leads to slowing of visual processing speed which underlies the overt clinical symptoms of simultanagnosia.


Poster

482. Alzheimer’s Disease: Risk Factors and Biomarkers

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 482.09/B78

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Alzheimer Foundation

Åhlén Foundation

Magnus Bergvalls Foundation

Uppsala Berzelii Centre

Title: Identification of exosomal proteins specifically released following Aβ1-42 protofibril treatment in co-cultures of primary neurons and glia

Authors: *E. NIKITIDOU1, P. EMAMI KHOONSARI2, G. SHEVCHENKO3, L. LANNFELT4, K. KULTIMA2, A. ERLANDSSON1;
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Abstract: Decreased Aβ1-42 and increased levels of tau/phospho-tau in cerebrospinal fluid (CSF) indicate biochemical changes typical for Alzheimer’s disease (AD). These biomarkers are a useful tool in AD diagnosis, but in order to follow disease progression and monitor drug intervention additional biomarkers, with a more dynamic expression pattern, are needed. Exosomes are small membranous vesicles formed by the invagination of the membrane around cytoplasmic materials. Exosomes have been implicated to have a pathogenic role in AD and it is likely that their content changes during the disease progression, making them a suitable source for biomarkers. The aim with the present study was to identify potential AD biomarkers, by comparing the content of exosomes and larger microvesicles released from primary neurons and glia in the absence and presence of Aβ1-42 protofibrils. Neural stem cells derived from embryonic mouse cortex were differentiated to a mixed culture of neurons, astrocytes and oligodendrocytes and exposed to Aβ1-42 protofibrils for 2 or 5 days, or left untreated. Uptake, degradation and toxicity were studied by immunocytochemistry and time-lapse microscopy. After clearing the sample from any remaining cell remnants, the culture medium was collected and ultracentrifuged to isolate exosomes and larger microvesicles. The presence of exosomes and larger microvesicles was confirmed by transmission electron microscopy (TEM) and their content was analyzed using mass spectrometry. Our data shows that astrocytes effectively engulf Aβ1-42 protofibrils, but store rather than degrade the ingested material. Aβ1-42 protofibrils did not directly induce cell death of neurons or glia, but time-lapse microscopy reveals that exposure of Aβ1-42 protofibrils results in extremely large astrocytic vacuoles, which are not seen in control cultures. The vacuoles appear to collapse and TEM analysis confirms that the cells secrete a variety of microvesicles, including exosomes. Exosomes were successfully isolated from the cell medium and display a disc shaped form. Mass spectrometry analysis revealed over 600 unique proteins that were expressed in exosomes and larger microvesicles. These proteins are involved in various cellular processes, including transcription, translation, inflammation and lysosomal degradation and some have previously been linked to AD. We have selected a few proteins that we consider to be of particular interest and we will next compare their expression in exosomes derived from CSF of AD patients and healthy controls.


Poster

482. Alzheimer's Disease: Risk Factors and Biomarkers

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM
Title: The effects of running on hippocampal vasculature and memory in a mouse model of amyloidosis

Abstract: Alzheimer’s disease (AD) is associated with cerebrovascular impairments including altered vascular density, increased tortuosity, and capillary fragmentation. Moreover, global hypoperfusion has been found to correlate with amyloid-β accumulation and exacerbated cognitive decline. Targeting the microvasculature 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 to prevent or delay some pathologies associated with AD, including vascular compromise. In addition, physical exercise has the potential to alleviate the burden associated with accumulation of cerebral amyloid angiopathy (CAA) on the vessel walls. We hypothesize that running has beneficial effects on brain microvasculature in a mouse model of amyloidosis. After developing plaque deposits and cognitive deficits, mice were placed for 3 months in a cage equipped with a spinning disk. After 3 months, animals were assessed for spatial memory with the Y-maze task. Subsequently, animals were injected with Methoxy-XO4 to visualize amyloid deposits. 24 hours post injection the animals were perfused with the 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 and amyloidosis 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 while CAA was manually segmented using the Imaris software. Pilot data suggest a decrease in capillary diameter and an increase in vessel count, volume and branching in non-running transgenic mice when compared to non-transgenic littermates. The above parameters derived for the vasculature in transgenic running animals were not statistically different when compared to non-transgenic
littermates. CAA surface area and volume were also significantly lower in running compared to non-running transgenic mice. Finally, spatial memory was maintained at higher levels in transgenic running mice compared to transgenic sedentary mice. The importance of vascular health in cognitive function for AD is receiving increasing interest. This research has the potential to provide new treatment options, including promotion of an active lifestyle to prevent or delay AD pathology, ultimately providing a better quality of life in the elderly population and in AD patients.


Poster

482. Alzheimer's Disease: Risk Factors and Biomarkers

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 482.11/B80

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: ARUK-PPG2012B-21

Title: Evaluation of 11C-BU99008, a radioligand for the imidazoline-2 binding sites, as a marker of reactive astrocytosis in a mouse model of Alzheimer's disease

Authors: *N. MIRZAEI, R. J. TYACKE, D. J. NUTT, M. SASTRE; Dept. of Med., Imperial Col. London, London, United Kingdom

Abstract: Amyloid-β (Aβ), which is the main component of neuritic plaques in Alzheimer’s disease patients, is believed to induce the activation of microglia and astrocytes in the vicinity of the amyloid deposits, leading to a neuroinflammatory response. Temporal changes in glial activation states are relatively unknown. Live imaging techniques such as positron emission tomography (PET) have enabled measurement and longitudinal monitoring of changes in microglial markers. We have recently developed a new PET tracer for astrocytes ([11C]2-(4,5-dihydro-1H-imidazol-2-yl)-1-methyl-1H-indole) ([11C]BU99008), to longitudinally monitor astrocytic activation. This ligand has a high affinity for imidazoline-2 binding sites (also known as I2-imidazoline receptors), which are expressed relatively selectively by astrocytes. We investigated the potential use of this tracer in a mouse model of amyloid pathology. Ex vivo binding studies were performed using [3H]BU99008 to characterize the distribution pattern and density of these receptors in 5xFAD mice (females; 6 months old) compared to aged-matched
wild-type controls by autoradiography. Higher $[^3]H$BU99008 binding was detected in the cerebral cortex and hippocampus of 5xFAD brains and more specifically in the subiculum and cortical layer 5. Interestingly, amyloid burden and the expression of astrocyte marker GFAP were significantly increased in the same areas as determined by immunohistochemistry. To further evaluate the regional distribution and cellular localisation of imidazoline receptors, we used two antibodies against Nischarin, a functional imidazoline receptor candidate. The Nischarin staining patterns co-localized with GFAP expression. In conclusion, these results strongly confirm the specificity of the radioligand $[^11]C$BU99008 as a potential marker of reactive astrocytosis and support its use as a human PET tracer for astrocytes.


Poster
482. Alzheimer's Disease: Risk Factors and Biomarkers

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 482.12/B81

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Korea government: the Ministry of Science, ICT & Future Planning (MSIP, No. 2010-0028631),

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Title: Disrupted rich club organization in Alzheimer’s disease and subcortical vascular dementia

Authors: *C. E. HAN*¹, H.-J. KIM¹, W. LEE², S. SEO², J.-K. SEONG¹;

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**Abstract:** The recent advances in magnetic-resonance (MR) imaging techniques and the graph theoretical analysis of the whole brain networks revealed that a few central brain regions are highly interconnected with each other than the others, called ‘rich clubs’ (van den Heuvel and Sporns 2011). This rich club organization has a crucial role in brain’s global communication, and thus was affected by various diseases (van den Heuvel et al. 2013, Ray et al. 2014). Though dementia significantly deteriorates the quality of life in the elderly, it is not well studied yet how dementia changes rich club organization (Daianu et al. 2013). In this study, we investigated rich club organization of patients with dementia; especially, its two common types, Alzheimer’s disease (AD) and subcortical vascular dementia (SVaD). Since their pathological hallmarks were distinct, we hypothesized that they exhibit different disruption pattern of rich clubs in the structural networks. We recruited 61 AD patients with AD, 39 patients with SVaD, and 36 age- and gender-matched normal controls (NC), and collected their T1-weighted and diffusion-weighted MR images using a 3-Tesla MR scanner at Samsung Medical Center. Institutional Review Board of Samsung Medical Center approved this study, and informed consent for participation was obtained from every participant. We constructed white matter brain networks using MR images, whose nodes are anatomically distinct brain regions defined by automated anatomical labeling (AAL) template (Tzourio-Mazoyer et al. 2002), and whose edges represent relationship between any pairs of two brain regions quantified through deterministic tractography (Mori and Barker 1999). We investigated rich-club organization of these structural networks following van den Huevel et al. (2011). We constructed group-averaged networks for NC, AD, and SVaD, and computed their rich-club coefficient normalized by 1000 degree-preserved random networks. Though all groups showed rich club organization, rich club coefficients of disease groups were higher than NC’s. While the rich club connections were mostly preserved, the local and feeder connections of disease groups reduced differently. The global efficiency was significantly decreased in both diseases but still strongly correlated with the rich club connections consistent with the literature (van den Heuvel et al. 2013). In conclusion, even with dementia, the structural network of brain follows the rich club organization and its rich club connections play a crucial role in global communication. Our study showed that AD and SVaD disrupt the rich club organization in different manners.

**Disclosures:** C.E. Han: None. H. Kim: None. W. Lee: None. S. Seo: None. J. Seong: None.

**Poster**

482. Alzheimer's Disease: Risk Factors and Biomarkers

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 482.13/B82
Comprehensive screening of amyloid-β aggregation inhibitors by a microliter-scale high-throughput screening system with quantum dot-based imaging technology

Title: Comprehensive screening of amyloid-β aggregation inhibitors by a microliter-scale high-throughput screening system with quantum dot-based imaging technology

Authors: *K. TOKURAKU, T. TAKAHASHI, Y. BABA, R. TAGUCHI, Y. HASHI, K. UWAI; Dept. of Applied Sci., Muroran Inst. of Technol., Muroran-Shi, Japan

Abstract: The amyloid hypothesis is widely known and accepted as the pathogenesis of Alzheimer’s disease (AD). In the hypothesis, the aggregation and accumulation of amyloid-β (Aβ) peptides in the brain is considered as the primary influence driving AD pathogenesis. Therefore, we believed that the Aβ aggregation inhibitors have a potential to become lead compounds for anti-AD agents. We recently reported a real-time imaging method of Aβ aggregation using quantum dot (QD) nanoprobes (Tokuraku et al., PLOS ONE 4, e8492, 2009). Subsequently, we developed a microliter-scale high-throughput screening (MSHTS) system for Aβ aggregation inhibitors based on the fluorescence imaging technology with QD nanoprobes (Ishigaki et al., PLOS ONE 8, e72992, 2013). This novel screening system could be analyzed with 5-µl sample volume, and estimated the activity of Aβ aggregation inhibitors as a half-maximal effective concentration (EC50). In this study, we tried to screen Aβ aggregation inhibitors by the MSHTS system from ethanolic extracts of about 100 kinds of plants containing land plants and seaweeds. The results revealed that almost more than 90% of the tested extracts showed inhibitory activity for Aβ aggregation, although the EC50 values were widely distributed (EC50: 0.001-10 mg/ml) among plant families. We also found that the ethanolic extracts of the Lamiaceae family showed significantly higher activity than the average of tested extracts, and an active compound in the Lamiaceae family was rosmarinic acid. Since the plants belonging Lamiaceae family, such as Salvia officinalis (sage) and Melissa officinalis (lemon balm), had already been reported to have memory-improving properties in old European reference books published hundreds of years ago, it is possible that the effect involves the inhibitory activity for Aβ aggregation of rosmarinic acid. Moreover, we also evaluated boiling water extracts of seaweeds by the MSHTS system. The results suggested that polysaccharides in the boiling water extracts of seaweeds affected morphology of Aβ aggregates and showed Aβ aggregation inhibitory activity.

Disclosures: K. Tokuraku: A. Employment/Salary (full or part-time); full. 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.; JSPS KAKENHI 25350974, JST A-STEP
Title: AD biomarker changes based on cortical thickness patterns

Authors: *J. ROH, J. HWANG, C. KIM;
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Abstract: Background: Neuropathologically defined subtypes of Alzheimer’s Disease (AD) have represented distinctive atrophy patterns and clinical characteristics in patients with AD. Cortical thickness based clustering of AD patients represented comparable results with autopsy findings culminating into 3 atrophy patterns: medial temporal (MT); diffuse (D); and parietal dominant (P) subtypes. It is not assessed, however, whether the new clustering method based on cortical thickness can reflect pathophysiologic changes in AD. Methods: A total of 77 AD subjects from the Alzheimer’s Disease Neuroimaging Initiative (ADNI) 2 dataset who completed 3T MRI, 18F-Fludeoxyglucose (FDG)-PET, 18F-Florbetapir-PET, and cerebrospinal fluid (CSF) study were enrolled for analyses. After obtaining cortical thickness using a CLASP algorithm, a hierarchical agglomerative cluster analysis was performed using a Ward’s clustering linkage to classify AD patients. FDG and Florbetabir PET data were compared among the groups using region of interest based analyses after controlling for age, gender, education, and intracranial volume. Differences in neuropsychological tests and CSF levels of amyloid-beta (Aβ) and tau were assessed among the groups. Results: Cortical thickness-based clustering methods demonstarated three patterns of cortical thinning in AD patients: MT subtype (19.5%), D subtype (55.8%), and P (24.7%) subtype. Patients in the P subtype (67.5±7.4 years) were younger than MT (74.8±7.9) and D (76.1±6.6) subtypes (p=0.0002). Patients in the P subtype represented more glucose hypometabolism in the left inferior parietal, right superior parietal, and left middle occipital cortices, which matches well with regions with cortical atrophy. Patients in the MT subtype revealed more glucose hypometabolism in left hippocampus and bilateral frontal cortices.
compared to the other 2 subtypes. Patients in the P subtype represented marked Aβ accumulation in most of brain regions, including superior, middle, inferior frontal cortices, superior, inferior parietal cortices, and precuneus measured by florbetapir-PET. Neuropsychological test results and levels of CSF revealed no difference among the groups. Conclusion: The findings that the P subtype represented more glucose hypometabolism and more accumulation of Aβ than the other groups and that the MT subtype had more glucose hypometabolism in compatible brain regions including the hippocampus indicates cortical thickness patterns can reflect pathophysiological changes in AD.

Disclosures: J. Roh: None. J. Hwang: None. C. Kim: None.

Poster

482. Alzheimer’s Disease: Risk Factors and Biomarkers

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 482.15/B84

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: A novel multiplex immunoassay enables detection of Alzheimer’s disease biomarkers in small volumes of cerebrospinal fluid

Authors: *L. CHEN, D. DROLL, A. J. SAPORITA, J. MISTRY, J. HWANG;
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Abstract: Alzheimer’s disease (AD) is a progressive neurodegenerative disorder that is the most common cause of dementia among the elderly. There are approximately 44 million people worldwide with dementia and AD, and it is the sixth leading cause of death in the U.S. Patients with AD suffer from memory loss and cognitive decline, which increases in severity as the disease progresses. Two key neuropathological features that exemplify AD are extracellular Amyloid β (Aβ) plaques and intracellular neurofibrillary tangles, which are composed of the abnormally hyperphosphorylated protein Tau. Biochemical changes in Aβ and tau reflect AD pathologic processes in the brain. Monitoring altered levels of these biomarkers in patient cerebrospinal fluid (CSF) may be highly beneficial to understanding disease progression. Using a MILLIPLEX® MAP multiplex immunoassay kit, simultaneous detection of Aβ1-40, Aβ1-42, total Tau, and phosphorylated Tau Thr181 in age-matched AD and non-AD human CSF samples was performed, thereby minimizing the use of valuable CSF samples (12.5 ul/well). Phosphorylated Tau and Aβ1-42 were significantly correlated to AD (p-value < 0.01 and p-value< 0.05, respectively). Neither total Tau nor Aβ1-40 showed a significant correlation in the CSF samples used in this study. The ratio of phospho-Tau Thr181 over Aβ1-42 improved the p-
value < 0.001. In addition, the specificity of this kit was tested and showed no cross-reactivity nor any significant difference in analyte concentrations regardless of whether each analyte was measured in a single-plex or multiplex (full 4-analyte kit) format. Furthermore, sensitivity, intra-and inter-assay precision, linearity of dilution and spike recovery exhibited excellent analytical performance. The MILLIPLEX® MAP Human Amyloid Beta and Tau panel provides a powerful tool for examining the pathogenesis of AD in both nonclinical and clinical research studies.


Poster

482. Alzheimer’s Disease: Risk Factors and Biomarkers

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 482.16/B85

Topic: C.05. Aging

Support: UWS Seed Grant to EG

Rebecca L Cooper Medical Research Foundation project grant to EG

Title: The effect of chronic neuroinflammation on cognition and the cholinergic system

Authors: *E. GYENGESI¹, A. RANGEL¹, O. KEKESI¹, P. YOON¹, K. V. K. GADALA¹, Y. BUSKILA², G. MUENCH¹;
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Abstract: Inflammation in the central nervous system and disruption of its immune privilege are major contributors to the pathogenesis of multiple neurodegenerative disorders, including Alzheimer’s (AD), Parkinson’s disease (PD), Creutzfeldt-Jakob disease (CJD), multiple sclerosis (MS), traumatic brain injury and more. Neuroinflammation and the loss cholinergic input from the basal forebrain to the hippocampus and the cortex have both been intensely associated with the development of dementia, and particularly AD, however the specific link between neuroinflammation and cholinergic cell death, and the interaction of cholinergic neurons and activated glia cells, is still an unexposed area. Interleukin-6 (IL6), among other proinflammatory cytokines such as IL-1 and TNF-α, is a common early inflammatory marker in AD and PD. Over the past decade, it has been shown that neuroinflammation plays a role in the development of neuronal degeneration, and as such, is a promising therapeutic target. In this study we used
heterozygous transgenic mouse GFAP-IL6, which express pro-inflammatory IL-6 in astrocytes under the control of the glial fibrillary acidic protein promoter; causing low level, chronic inflammation in the brain. In this study, we have used heterozygous GFAP-IL6 mice to study motor-coordination, anxiety and cognitive performance together with immunohistochemistry. At 3 months of age, GFAP-IL6 mice showed decreased performance on rotarod, elevated narrow beam walk, hind limb clasping and ledge tests as well as in novel object recognition memory, indicating deficits in fine motor coordination that continued to deteriorate with age, reaching significant decline at 22 months of age. The behavioral changes were accompanied by increased microglia and astrocytes numbers and activation throughout the brain, and decreased cholinergic cell numbers in the basal forebrain at 22 months of age. These results confirm that the GFAP-IL6 transgenic mouse displays subtle deficits in fine coordination and memory already at 3 months of age that gradually worsens throughout the life span. Our results suggest that chronic neuroinflammation could potentially be a significant distributor to motor and cognitive function decline throughout the aging process. This mouse model may suitable to test anti-inflammatory medications for the treatment of AD and PD.


Poster

482. Alzheimer's Disease: Risk Factors and Biomarkers

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 482.17/B86

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Chancellor’s Innovation Fund: Proof of Concept Award

NIH P01AG030128-06

Title: Development of novel Alzheimer's disease plasma biomarkers: oligomeric Aβ and apoE/Aβ complex

Authors: *N. C. COLLINS, S. GHURA, K. P. KOSTER, M. LADU; Anat. and Cell Biol., Univ. of Illinois At Chicago, Chicago, IL

Abstract: Alzheimer’s disease (AD) is a rapidly growing epidemic with a conservative predicted incidence of 14 million Americans by 2050, at a cost of $1 trillion. Despite these alarming statistics and the emotional devastation caused by AD. There are no biological biomarkers for
AD that are predictive or reliably diagnostic, or based on the underlying causes of AD. Clinical trials amplify this issue, as without mechanistic biomarkers, it is virtually impossible to identify novel preventative drugs, or track drugs designed for treatment of AD. Amyloid-beta (Aβ) peptide is a major cause of cognitive decline in AD. Aβ self-aggregates to form oligomeric Aβ (oAβ), considered a proximal neurotoxin in AD. The gene encoding apolipoprotein E4 (APOE4) is the greatest genetic risk factor for AD. APOE4 increases risk up to 15-fold, compared to the more common APOE3 genotype, while APOE2 reduces risk 4-fold compared to APOE3. Evidence suggests that one mechanism by which the apoE isoforms modulate AD risk is through the modulation of Aβ levels, an effect potentially mediated by physical interactions between apoE and Aβ (apoE/Aβ complex). Through an intensive research program, we developed a unique monoclonal antibody, MOAB-2, that enabled development of assays that detect oAβ and apoE/Aβ complex. Previous studies demonstrated that in both human brain and CSF, the levels of oAβ are higher with AD compared to control, and in patients with AD, higher with APOE4 compared to APOE3. Importantly, levels of apoE/Aβ complex are lower in AD patients compared to control, and within AD patients, apoE4/Aβ levels are lower than apoE3/Aβ levels. Thus, our hypothesis is that oAβ and apoE/Aβ complex levels are mechanistic biomarkers that track AD progression based on APOE genotype. A major obstacle for measurement of CSF biomarkers are CSF draws, which are highly invasive and do not allow for the repeated measures necessary for both diagnosis and theragnosis. Thus, an ideal biomarker would be both highly sensitive and selective allowing for measures in human plasma. Therefore, we measured oAβ and apoE/Aβ levels in human plasma. Our data in human plasma demonstrates that oAβ levels are higher and apoE/Aβ complex levels are lower in AD compared to control and with APOE4 compared to APOE3. Collectively, our results support the hypothesis that APOE modulated changes in apoE/Aβ and oAβ levels underlie the APOE4-induced risk for AD. Further, plasma levels of apoE/Aβ and oAβ may represent mechanistic biomarkers for the prediction and diagnosis of AD.


Poster

482. Alzheimer's Disease: Risk Factors and Biomarkers

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 482.18/B87

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: R01 AG037639
Title: Insulin resistance and APOE ε4 allele status are linked with altered myelin in asymptomatic middle-aged adults

Authors: *J. P. O'GRADY¹, C.-M. CANDA², D. C. DEAN, III³, J. SOJKOVA², E. J. STARKS², S. HURLEY⁵, N. J. DAVENPORT², O. C. OKONKWO², S. ASTHANA², M. A. SAGER², S. C. JOHNSON²,4,6, A. L. ALEXANDER³, B. B. BENDLIN²,4;

Abstract: Background: Recent analyses suggest that insulin resistance (IR) is associated with several brain changes including lower regional volumes, lower glucose uptake, lower cerebral blood flow, and Alzheimer's disease (AD) pathology as measured in cerebrospinal fluid, as early as midlife. Still, little is known about the impact of insulin and IR on cerebral white matter. Objective: The aim of this study was to determine the effect of IR on myelin in healthy late-middle-aged adults. Given the role of insulin in cholesterol synthesis, we hypothesized that IR would be associated with a negative effect on myelin as shown on a novel brain imaging marker of myelin content. Methods: Asymptomatic adults enriched for risk factors for AD (N=127, mean age = 61.8 years) from the Wisconsin Registry for Alzheimer's Prevention study (WRAP) underwent Multi-component Driven Equilibrium Single-Pulse Observation of T1 and T2 (mcDESPOT) imaging on a GE MR750 3T scanner. Fasting blood draw was used to determine IR using the Homeostatic Model Assessment for IR (HOMA-IR). A linear regression analysis tested the effect of IR, APOE ε4 status, and the potential interaction between APOE ε4 and IR on myelin water fraction (MWF) maps using SPM12 software. Results: HOMA-IR was associated with altered myelin, shown by a main effect of HOMA-IR on MWF. This main effect was explained by a significant interaction between APOE ε4 and HOMA-IR. Higher HOMA-IR was associated with lower MWF, but only among APOE ε4 carriers. Conclusion: The results suggest that IR is associated with myelin content, and the effect is moderated by APOE ε4 genotype. Interestingly, higher IR was associated with lower MWF, but only among APOE ε4 carriers. Given that APOE ε4 and IR are risk factors for AD and their physiology converges on lipid processing, the current results suggest that negative effects on myelin may play a role in vulnerability to AD.

**Title:** CSF biomarkers of the neurovascular unit and the impact of APOE4 genetic risk in mild Alzheimer’s disease

**Authors:** *M. D. Sweeney*¹, A. P. Sagare¹, D. A. Nation¹, M. R. Halliday¹, A. M. Fagan², J. C. Morris², B. V. Zlokovic¹; ¹USC, Los Angeles, CA; ²Dept. of Neurol., Washington Univ. Sch. of Med., St. Louis, MO

**Abstract:** Vascular dysfunction is increasingly recognized in the etiology of Alzheimer’s disease (AD). Established biomarkers, amyloid-β (Aβ) and tau, do not predict the onset of cognitive decline and thus have limited clinical use. A clinical need exists to identify reliable biomarkers for early AD diagnosis, identifying novel treatment targets, and evaluating the effectiveness of clinical trials. Thus, we quantified novel cerebrospinal fluid (CSF) biomarkers of responses/injury to the neurovascular unit (NVU) - comprising vascular cells, glia, and neurons - using antibody-based single/multiplex assays. CSF biomarkers of the NVU in human subjects were analyzed by the degree of cognitive impairment, assessed via the Clinical Dementia Rating (CDR) scale, and the influence of apolipoprotein E-ε4 (APOE4), the major genetic risk factor for late-onset AD. We observed that CSF biomarkers of blood-brain barrier (BBB)/vascular injury were altered during early stages of cognitive impairment prior to inflammatory, neuronal, and Aβ biomarkers. APOE4 carriers but not non-carriers exhibited an age-dependent increase in albumin CSF/plasma ratio. Also, albumin ratio increased 55% in CDR 0.5 compared to CDR 0 in APOE4 carriers, and correlated significantly with CSF fibrinogen levels in APOE4 carriers with CDR 0.5. CSF levels of soluble platelet-derived growth factor receptor-β (sPDGFRβ), a marker of pericyte injury, increased by 60% and 27% in APOE4 carriers and non-carriers, respectively, between CDR 0 and CDR 0.5 groups. Additionally, CSF biomarkers of endothelial cells (i.e., adhesion molecules) correlated with BBB breakdown only in APOE4 carriers. CSF levels of
inflammatory cytokines and neuronal injury markers (i.e., total and phosphorylated tau and neuron specific enolase) were not altered in subjects with CDR 0, 0.5, or 1, regardless of APOE genotype. Finally, CSF Aβ42 levels were 28% lower in APOE4 carriers compared to non-carriers at CDR 0.5 and decreased by 60% in APOE4 carriers from CDR 0.5 to 1. These data reveal that CSF biomarkers of the NVU are differentially altered in early cognitive impairment and influenced by APOE4, beginning with changes in BBB/vascular injury biomarkers. Overall, this suggests that a molecular algorithm may define stages of cognitive impairment and AD development and could importantly aid in AD-specific diagnostic and treatment efforts.

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Poster

482. Alzheimer's Disease: Risk Factors and Biomarkers

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 482.20/B89

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIA P50 AG05146

NIA RC2 AG36419

T32 AG027668

Title: The role of ApoE-4 in hippocampal hyperactivity in amnestic mild cognitive impairment

Authors: *T. TRAN*, C. SPECK, A. PISUPATI, M. GALLAGHER, A. BAKKER; ¹Psychological & Brain Sci., Johns Hopkins Univ., Baltimore, MD; ²Johns Hopkins Sch. of Med., Baltimore, MD

Abstract: Increased fMRI activation in the hippocampus is recognized as a signature characteristic in the aMCI stage of Alzheimer’s disease (AD) and a predictor of cognitive decline and progression to AD dementia. Recent studies have localized this increased activation to the dentate gyrus / CA3 subregion of the hippocampus and showed a correlation with memory impairments in those patients, as predicted by a rodent model of aging. Increased hippocampal activation has also been reported in carriers of the ApoE-4 allelic variation independently of mild cognitive impairment although these findings were not localized to a hippocampal sub region.
Animal models of ApoE-4 predict that this increased activation would be similarly localized to the dentate gyrus / CA3 region. To assess whether ApoE-4 contributes to increased hippocampal fMRI activation, 62 patients with aMCI genotyped for ApoE-4 status and 30 healthy age-matched control participants completed a high-resolution fMRI scan while performing a memory task designed to tax hippocampal sub region specific functions as well a neuropsychological assessment. Consistent with previous reports, patients with aMCI showed increased hippocampal activation in the left dentate gyrus / CA3 sub region of the hippocampus as well as memory task errors attributable to this subregion of the hippocampus. However, this increased fMRI activation in the hippocampus did not differ between ApoE-4 carriers (N = 25) and ApoE-4 non-carriers (N = 37) and the proportion of memory errors attributable to dentate gyrus / CA3 function did not differ between ApoE-4 carrier and ApoE-4 non-carriers. These results indicate that increased fMRI activation of the hippocampus observed in patients with aMCI is independent of ApoE-4 status and that ApoE-4 does not contribute to the dysfunctional hippocampal activation or the memory errors attributable to this sub region in these patients.

differential lymphocyte proliferation in response to mitogenic stimulus. Multivariate algorithms have shown that the LymPro Test can distinguish AD from HN in clinically diagnosed cohorts with 80% accuracy or greater. This study reports the effect of ApoE4 and MMSE score on the LymPro Test. The LymPro Test potentially represents a useful blood-based biomarker for AD.

Methods: 140 blood samples were obtained and 125 of the results passed analytical review (59 AD and 66 controls). Samples were analyzed (Becton Dickinson) for CD69 frequency (a surface marker of cell cycle activity) on peripheral T, B, and monocyte cells by flow cytometry with and without mitogenic stimulation. Each subject had ApoE allele determination (Quest labs) and AUC point estimates were determined for all LymPro flow variables with ApoE4+ AD compared to ApoE4- HN. MMSE scores were also compared on a univariate and multivariate basis with results compared within and between cohorts. Results: Multiple univariate measures were significantly (p<0.05) different in AD subjects compared with HN subjects. ApoE4 allele generally had little effect on univariate measures. A multivariate algorithm AUC score slightly declined when ApoE4- AD subjects were excluded from the analysis population. LymPro multivariate result showed no significant change when AD subjects were compared within cohort. Mild, moderate and severe AD cohorts were not statistically different on a key univariate measure. Conclusions: ApoE4 allele presence did not appreciably affect the LymPro results, indicating likely independence from this genotype. AD severity as measured by MMSE had little effect on the LymPro results suggesting insensitivity to dementia severity. This indicates that LymPro Test likely will show a signal in prodromal AD. Further research is planned.

Disclosures:  L. Kirby: F. Consulting Fees (e.g., advisory boards); Amarantus Diagnostics. P. Jorgensen: F. Consulting Fees (e.g., advisory boards); Amarantus Diagnostics. D.A. Lowe: F. Consulting Fees (e.g., advisory boards); Amarantus Bioscience Holdings, Inc.. Other; Industry Funded Trial (Amarantus Bioscience Holdings, Inc.). C. Bier: F. Consulting Fees (e.g., advisory boards); Amarantus Diagnostics. M. Sabbagh: 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.; Banner Sun Health Research Institute.

Poster

482. Alzheimer's Disease: Risk Factors and Biomarkers

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 482.22/B91

Topic: C.05. Aging

Support: NIA U01 AG031115 to RDB
Title: IND-enabling acute and chronic preclinical safety and pharmacokinetics of allopregnanolone regenerative treatment regimen for Alzheimer’s disease

Authors: *R. W. IRWIN*, C. M. SOLINSKY, K. KIM, C. GREEN, G. BAUER, M. A. ROGAWSKI, K. E. RODGERS, R. D. BRINTON;  

Abstract: We aim to develop allopregnanolone (Allo) as the first regenerative therapeutic for Alzheimer’s disease. Dose escalation and range finding studies were performed prior to selection of doses for chronic exposure. Allo formulated in cyclodextrin at 1:6 complexation resulted in optimal rate and efficacy of Allo delivery to brain by intramuscular route of administration and advanced to chronic exposure studies. Safety studies were conducted to identify target organs of toxicity and to estimate the no observed adverse effect level (NOAEL) of Allo after 24 weeks of once-per-week dosing to adult male and female Sprague Dawley rats. Rats were bolus administered Allo 0, 1, 4, and 8mg/kg intramuscularly once-per-week for 24 weeks. Necropsies were on Day163 (Main group) and on Day191 (Recovery group). Endpoints were clinical observations, body weight, food consumption, functional observational battery (FOB), ophthalmologic examination, plasma drug levels and toxicokinetics, clinical pathology, urinalysis, organ weight, and macroscopic and microscopic tissue examination. No toxicologically relevant changes were found in body weight, food consumption, ophthalmology, gross necropsy, organ weight, urinalysis parameters, or clinical pathology. Acute sedative effects were observed in high-dose groups determined by FOB parameters (home cage, handling, and reflex and physiology measurements) primarily in high-dose males and females on Day1 and 162. Sedative effects for high-dose groups were enhanced in males vs females. The incidence of epithelial hyperplasia in the non-glandular stomach was increased in highest dose Main group males but was not found in Recovery group. Allo blood levels were similar between males and females on Day1 at all doses, and trended higher in males compared to females on Day162 in the mid- and high-dose groups. Day162 exposures trended higher than Day1 at all doses. Terminal phase half-life values were less than 1hr in all groups except high-dose males on Day162. All animals survived to the end of the study. At 4 and 8mg/kg dose levels, threshold of sedation was achieved based on behavioral observations related to sedation. The high-dose group males exhibited histopathological changes to the nonglandular stomach, an anatomical feature not present in humans. Based on these findings, the MTD for Allo is ~4mg/kg, and the NOAEL is ~1mg/kg. NOAEL of 1mg/kg intramuscular injection is equivalent to 0.19 mg/kg in humans. Clinical dose-escalating studies with Allo (ClinicalTrials.gov Identifier: NCT02221622) are
warranted to determine safety and tolerability. Research supported by NIA U01AG031115; NIA U01AG047222; and UF1AG046148 to RDB.


Poster

482. Alzheimer’s Disease: Risk Factors and Biomarkers

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 482.23/B92

Topic: C.05. Aging

Support: NIA 5P01AG026572

Title: The role of perimenopause, inflammation and apoe4 in the pathogenesis of Alzheimer’s disease

Authors: *A. MISHRA*¹, F. YIN², A. CHRISTENSEN³, C. J. PIKE³, E. CADENAS², R. D. BRINTON²; ¹Clin. therapeutics, ²Pharmacol. and Pharmaceut. Sci., ³Davis Sch. of Geronotology, USC, Los Angeles, CA

Abstract: Alzheimer’s disease (AD) is known to have a long latent prodromal stage, before the symptoms and the pathology actually manifest. The etiology of the disease, obscured by its multifactorial nature, poses a challenge in isolating the effects of each risk factor and studying the mechanism involved in the development of Late Onset Alzheimer’s disease (LOAD). The perimenopausal transition in women, characterized by symptoms of hot flashes, insomnia, fatigue, marks the beginning of reproductive senescence causing a reduction in the levels of female reproductive hormones- estrogen and progesterone. The perimenopausal transition is considered a “tipping point” in the prodromal stage of AD. NSAIDs (Non steroidal anti inflammatory drugs) on the other hand have been known to delay the onset of LOAD, and are shown to be most effective in their preventive action when taken during the ages of 40-50 years. The overlap in the age group undergoing the perimenopausal transition and the age group more susceptible to the preventive action of NSAIDs, indicates a correlation between the two factors and how they would be affecting the AD pathogenesis in the prodromal stage. Estrogen is a potent anti-inflammatory agent, and perimenopause causes a depletion of this hormone leading to a consequent increase inflammation both peripherally and centrally. In our preliminary study in the perimenopausal rat model which isolates endocrine aging from chronological aging, the
gene expression profiling of hippocampus and cortex indicated an upregulation of genes involved in the TLR4/MyD88/NFkB pathway in the irregular cycling and acyclic rats in comparison to regular cycling rats of the same age. Serum cytokine profiling in the same rats indicated a significant increase of IL-12 and IL-1A in acyclic rats as compared to regular cycling rats of the same age. This increase in proinflammatory cytokines correlated inversely with the levels of 17-B estradiol in plasma. On the basis of these preliminary analyses, we aim to characterize the inflammatory phenotype with respect to the perimenopausal transition and how both these factors together affect the pathogenesis of LOAD. Another key aspect of our analyses will include a focus on the effect of APOE4 allele on the inflammatory phenotype exhibited in the perimenopausal transition. Using the humanized ApoE4 KI rats and mice as our model, we aim to isolate the effect of this allele on the perimenopausal transition and how inflammation would be supplementing its effect in the progression of AD. This work was supported by NIA 5P01AG026572 to RDB; Project 1 to RDB & EC.


Poster

482. Alzheimer’s Disease: Risk Factors and Biomarkers

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 482.24/B93

Topic: C.05. Aging

Support: NIA 5P01AG026572

Title: APOE4 impairs cognitive performance in female APOE targeted-replacement and EFAD mice

Authors: *A. CHRISTENSEN¹, C. J. PIKE²;
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Abstract: Alzheimer’s disease (AD) is more prevalent in women than men. The reasons for this are still unclear, but are not simply due to the longer mean lifespan of women. One contributing factor may be the depletion of estrogens and progesterone at menopause. Estrogens in particular have been shown to exert several neuroprotective actions relevant to AD in multiple rodent paradigms. For example, estrogens decrease inflammation and reduce the incidence of metabolic disorders, both of which have been linked to greater AD risk. Another AD risk factor that exhibits sex differences is the ε4 allele of apolipoprotein E (ApoE4), the major genetic risk factor
for non-familial AD. ApoE4 confers significantly greater AD risk in women than in men. Thus, the combination of estrogen loss and ApoE4 status may contribute to the vulnerability of women to AD. To investigate this issue, we studied the independent and combined effects of ApoE4 and estrogen on cognitive and AD-like neuropathology in female mice with and without depletion of endogenous estrogen. We employed female mice with targeted replacement of humanized ApoE3 and ApoE4 (TRE3, TRE4; P. Sullivan) as well as targeted replacement ApoE mice that were crossed with the 5xFAD transgenic model (EFAD; M.J. LaDu). Targeted replacement (TRE3, TRE4) and EFAD (E3FAD, E4FAD) female mice were ovariectomized (OVX) or sham OVX at three months of age. OVX removes the peripheral source of sex steroid hormones. Sixteen weeks after surgery, mice were evaluated for behavioral performance then euthanized and assessed for AD-related neuropathology. Female TRE4 mice performed more poorly than TRE3 mice on both the novel object recognition (NOR) and novel object placement (NOP) tasks. OVX was associated with poorer performance on NOP but not NOR. EFAD mice showed similar behavioral trends: E4FAD females performed worse than E3FAD females on NOP and NOR but only NOP showed worsening performance with OVX. β-Amyloid (Aβ) plaques are a hallmark of AD neuropathology and are present in the EFAD mice. We observed an increase in Aβ burden in E4FAD females compared to E3FAD females, but no further increase by OVX. Thus, ApoE4 is associated with poorer cognition in both the presence and absence of AD-related neuropathology. Depletion of endogenous estrogens does not yield worsened Aβ burden but has mixed effects on behavioral outcomes. The modest effects of estrogen depletion on deleterious outcomes of ApoE4 suggest that the increased AD risk in women associated with ApoE4 is likely independent of estrogen’s activational effects, suggesting an important hormone independent action.

Disclosures: A. Christensen: None. C.J. Pike: None.

Poster

482. Alzheimer's Disease: Risk Factors and Biomarkers

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 482.25/B94

Topic: C.05. Aging

Support: NIA P01AG026572 (RDB): Project 2 (CEF), Project 3 (CJP)

Title: Sex and APOE in the amyloid burden and microbleeds of EFAD mice and humans

Authors: *M. CACCIOTTOLO1, A. CHRISTENSEN1, A. ALEXANDRA MOSER1, J. LUI1, C. J. PIKE1, T. E. MORGAN1, E. BACON1,2, G.-Y. CHIANG3, C. E. FINCH1,2;
Abstract: Women APOE4 carriers have the highest risk for Alzheimer's disease (AD), but sex-APOE allele interactions are unclear for AD neuropathology. To further resolve APOE-sex interactions, we examined EFAD mice transgenic for familial AD genes together with targeted replacement (APOE-TR) human APOE3 and -E4 alleles (Youmas et al, 2012) for endpoints of Aβ burden, cerebral amyloid angiopathy (CAA) and microbleeds. In EFAD mice at age 7 months, female sex and APOE genotype independently increased oligomeric Aβ42 and total PBS extracted Aβ (2- to 8-fold, F>M, ELISA). Female EFAD also had 2-fold larger Aβ plaques (with modest increase by apoE4). Cerebrovascular pathology followed these trends of female excess and APOE4: for CAA, the number of Aβ-positive microvessels differed 2-fold by APOE allele, which was increased 50% in females of both alleles. The microbleeds (microhemorrhages) were in >5-fold female excess with non-significant differences by APOE. The background APOE-TR mice had 20-fold fewer microbleeds than EFAD, with 2-fold female excess. Microbleeds may be precursors of gross lobar hemorrhages in APOE-TR mice by 18 months (Sullivan et al, 2008). Because aging wildtype mice do not develop microbleeds or lobar hemorrhages, these findings suggest that the introduction of human APOE3- or 4 interacts with endogenous murine Aβ to cause cerebrovascular pathology which is underappreciated. Sequence differences between human and mouse APOE and APP may underlie these vascular phenotypes. Human APOE-sex interactions were further explored in the Alzheimer’s Disease Neuroimaging Initiative (ADNI), which showed the opposite sex bias, with male APOE4 carriers having more microbleeds, again with APOE4 dose effect. These findings suggest species differences in the neurobiology of APOE-sex interactions, relevant to AD as a uniquely human condition (Finch and Austad, 2015).

Abstract: APOE4 carriers, particularly women, have the highest risk for Alzheimer's disease (AD), but sex-APOE allele interactions are unresolved. Because of the importance of inflammatory processes during AD, APOE-sex interactions for glial density and cytokines were analyzed in the EFAD mouse model. EFAD mice are transgenic for familial AD genes together with targeted replacement (APOE-TR) human APOE3 and -E4 alleles (1). At age 7 months, EFAD mice show independent effects of female sex and APOE4 for oligomeric Aβ and as plaques in cerebral cortex (2). We further show that female EFAD mice have 20-50% higher glial density, for microglia (Iba-1) and astrocytes (GFAP), with stronger APOE4 effects for microglia in females. Cytokine levels showed a range of associations with APOE alleles and sex. Often cytokines, only IL-6 and TNFa differed by APOE (E4>E3, 50-100%), while these and others were higher in females (IL1β, IL4, IL6, IL10; KC/GRO and TNFa). No sex or APOE allele differences were found for IFNγ, IL2, IL5, or IL12p70. The absence of APOE effect for most of the analyzed cytokines in EFAD male mice confirms findings on male APOE-TR (3). The EFAD mouse model suggests that APOE-sex interactions are restricted to subset of inflammatory cytokines that includes IL6 and TNFa. Both IL-6 and TNFa are recognized in AD inflammatory processes mediated by microglia, which also showed the strongest sex-APOE interaction. We hypothesize that the greater AD risk of women APOE4 carriers involves their higher microglial load and levels of a subset of cytokines including IL-6 and TNFa. We suggest human responses to anti-inflammatory drugs might be informed by sex-APOE interactions.


Disclosures: T.E. Morgan: None. C.E. Finch: None. M. Cacciottolo: None.

Poster

482. Alzheimer's Disease: Risk Factors and Biomarkers

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 482.27/B96

Topic: C.05. Aging

Support: NIA U01 AG031115
Title: Preclinical safety and efficacy of allopregnanolone in a mouse model for Alzheimer’s disease

Authors: *C. C. CALDWELL1, R. W. IRWIN2, A. ROMANI3, C. SOLINSKY1, S. CHEN2, R. D. BRINTON2,4; 1Clin. and Exptl. Therapeut., 2Pharmacol. and Pharmaceut. Sci., USC Sch. of Pharm., Los Angeles, CA; 3Dept. of Biomed. and Specialist Surgical Sci., Univ. of Ferrara, Ferrara, Italy; 4Neurol., Keck Sch. of Medicine, USC, Los Angeles, CA

Abstract: Allopregnanolone (Allo) promotes regeneration, associative learning and memory, and reduces β-amyloid burden in triple transgenic Alzheimer’s disease (3xTgAD) mice. To further develop allopregnanolone as a therapeutic for Alzheimer’s disease, we investigated once per week administration in male and female 3xTgAD animal model. Adverse outcomes of clinical trials with immunotherapeutic agents that target β-amyloid led FDA to institute the precaution to investigate risk potential for cerebral microhemorrhage in a preclinical model. Although Allo is not an immunotherapy against β-amyloid, Allo does decrease β-amyloid load in brain. 19-23 month-old 3xTgAD mice were given once weekly subcutaneous injection of vehicle or allopregnanolone (6 mg/kg) or intraperitoneal injections of 3D6 (antibody dosed at 50 mg/kg) for a period of 6 weeks. Each treatment group was composed of 10-12 females and 5-7 males 3xTgAD transgenic mice. In male and female 3xTgAD mice, formulations were tested at maximally tolerated doses by subcutaneous route assessed by Perls stain to detect cerebral amyloid angiopathy-associated microhemorrhage. Quantitative analyses indicated no elevation in microhemorrhages in Allo treated mice when compared to vehicle. Our previous data demonstrated that neuro-regeneration, maintenance of cholesterol homeostasis, and reduction of AD pathology were achieved with once per week Allo treatment. Histology and cytochemistry of brain sections from aged 3xTgAD mice dosed with either Allo or vehicle control were immunolabeled for the following pathology markers: β-amyloid, PHF-Tau; Iba-1 activated microglia; ABAD amyloid peptide binding protein on mitochondria; and CNPase, a oligodendrocyte marker for labeling myelin structure of white matter in brain. Images were analyzed both by qualitative pathology structure and location and by quantitative stain intensity. Analyses indicated that Allo treated mice exhibited a reduction in β-amyloid plaque size and prevalence. Iba-1 staining showed a strong association of activated microglia surrounding β-amyloid deposits and tau tangles. Additional trends of decreased Tau, ABAD, and increased white matter were associated with Allo treatment. These data support the development of Allo as a therapeutic candidate for Alzheimer’s disease. Further analyses are in progress. This study has been supported by NIH National Institute on Aging NIA U01 AG031115 and NIA U01 AG047222 to RDB.

Title: Allopregnanolone and its analogues differentially potentiate mitochondrial function and gene expression in human neural stem cells

Authors: *M. DESAI*, R. W. IRWIN, K. GEE, R. D. BRINTON;  

Abstract: Allopregnanolone (Allo) is in clinical development as the first regenerative therapeutic for Alzheimer’s disease and other neurological disorders. In this study we sought to determine the action of Allo and analogues thereof on mitochondrial function of human neural stem cells (hNSCs) and then examine the gene expression profile responsible for differential mitochondrial potentiation. These in vitro hNSC studies were designed to assess potential biomarkers of Allo efficacy that can be translated to studies on patient derived stem cells. Mitochondrial function was tested using the Extracellular Flux Analyzer manufactured by Seahorse Biosciences. First we determined the optimum cell density and Allo concentration to be used. Treatment of hNSCs with Allo resulted in an inverted U-shaped dose response curve with the peak response observed at 100nM that was consistent with earlier published reports. Maximal respiration and spare respiratory capacity of hNSCs were increased by 39% and 53% respectively after treatment with Allo for 24h, compared to vehicle. Additionally, we conducted a pulse-based experiment to determine activation time requirement of hNSCs. The cells were treated with a 30 min pulse of Allo and mitochondrial function of hNSCs determined at multiple time points post-treatment. The pulse treatments were compared to a 24-hour continuous Allo treatment. We determined that 30 min pulse Allo administered 8 hours or 4 hours prior to measurement potentiated the mitochondrial function of hNSCs to a level equivalent to 24-hour continuous Allo treatment. This further strengthens our translational research-based decision to administer Allo intravenously over 30 min. for Phase I clinical trial for MCI and early AD
(ClinicalTrials.gov Identifier: NCT02221622). We are currently evaluating mitochondrial gene expression levels in hNSCs in response to Allo treatment and expect to find that all 13 mitochondrial genes are upregulated by Allo. This study has been supported by NIH National Institute on Aging U01-AG047222 and UF1-AG046148 to RDB; USC Provost Fellowship to MKD

**Disclosures:** M. Desai: None. R.W. Irwin: None. K. Gee: None. R.D. Brinton: None.

**Poster**

**482. Alzheimer's Disease: Risk Factors and Biomarkers**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 482.29/B98

**Topic:** C.05. Aging

**Support:** NIH NIA Grant U01AG031115

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Donald E. and Delia B. Baxter Foundation Grant

NIH NIA AG005142

USC Provost Fellowship

CIRM Predoctoral Research Traineeship

**Title:** Developing mitochondrial function and regenerative potential as biomarkers of patient response to allopregnanolone treatment

**Authors:** *C. M. SOLINSKY*¹, M. K. DESAI¹, V. HENNES², H. C. CHUI³, J. K. ICHIDA², R. D. BRINTON¹;

¹Clin. Pharm. and Pharmaceut. Econ. & Policy, ²Stem Cell Biol. & Regenerative Med., ³Neurol., USC, Los Angeles, CA

**Abstract:** Alzheimer’s disease (AD) is a national and global epidemic with complex pathoetiology including compromised brain metabolic activity and decreased regenerative capacity. Allopregnanolone (Allo) is an investigational neuroregenerative therapeutic, currently in Phase 1b clinical trial for AD (NCT02221622,
In rodent preclinical models, Allo promotes neural stem cell (NSC) proliferation and neural differentiation and improves mitochondrial function. To develop biomarkers to predict regenerative response to Allo, we have initiated proof of concept analyses to determine the impact of Allo on human lymphocyte derived induced pluripotent stem cells (iPSCs) and iPSC-derived neural cells. T-cells from a patient with familial AD due to the A431E presenilin-1 (PSEN1) point mutation were reprogrammed to iPSCs. Using dual inhibition of SMAD signaling A431E iPSCs were differentiated to NSCs. Mitochondrial respiration and regenerative capacity were determined using metabolic analyzer, High Content Screening imaging, and FACS. Mitochondrial respiration analyses demonstrated that in PSEN1 mutation containing iPSC-derived NSCs treated with Allo 100nM, ATP turnover increased by 120% and maximal mitochondrial respiratory capacity by 100%. Concurrently, analyses were conducted to determine the regenerative effect of Allo on primary human NSCs. In human NSCs, Allo increased basal mitochondrial respiration by 25% and maximal mitochondrial respiratory capacity by 37.5%. FACS analysis indicated that Allo increased the number of Ki67 positive cells by 12% and, within that population, a rise in expression by 52%. Initial data demonstrate that iPSCs derived from PSEN1 lymphocytes can be generated, differentiated to NSCs, and their metabolic phenotype determined. Data indicate that Allo can increase mitochondrial respiration and promote regeneration of human-derived NSCs. Going forward the effect of Allo on the regenerative capacity and metabolic phenotype of PSEN1 mutation containing and sporadic iPS-derived NSCs will be evaluated. These data will form the foundation for developing the first regenerative biomarker to determine and monitor response to therapeutics. Research supported by NIH National Institute on Aging U01AG031115 and UF1AG046148 to RDB; NIH National Institute for Neurological Disorders and Stroke R00-NS07743 and the Donald E. and Delia B. Baxter Foundation to JKI; NIH National Institute on Aging AG005142 to HCC; USC Provost Fellowship, CIRM Predoctoral Research Traineeship, and American Foundation for Pharmaceutical Education Fellowship to CMS.


Poster 483. Alzheimer's Disease: Tau

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 483.01/B99

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Fine-mapping of the MAPT gene variation in progressive supranuclear palsy
Abstract: Multiple association studies, including genome-wide association studies, have shown that MAPT H1 haplotype is associated with risk of developing progressive supranuclear palsy (PSP), more specifically; MAPT subhaplotype H1C is highly associated to PSP. However, the precise causal variants at the MAPT locus are still unknown. We performed a comprehensive association study of the locus to fine-map the association signal and identify causal variants in PSP. First, we used MAPT tag SNPs (rs1467967, rs242557, rs3785883, rs2471738, rs8070723, rs7521) to identify the associated H1 subhaplotypes in our large pathologically-confirmed series of 917 PSP cases and 974 healthy controls. We confirmed the association to H1C and report new PSP associated H1 subhaplotypes (H1G, H1D, H1O and H1I). We followed this step by a targeted re-sequencing of the entire MAPT gene including 10kb upstream and downstream using genome capture and next generation sequencing. This screening stage was performed on 280 PSP samples and 280 controls. After, quality control we completed association tests on common SNPs (Minor allele frequency>1%) and identified 30 variants (P<0.05) for follow-up. We validated and genotyped these variants in our PSP cases/controls total series (~2000 subjects). We identified three intronic variants associated to PSP: one located in intron 1, one in intron 10 and one downstream MAPT. The intron 1 and downstream variants sit on the H1C subhaplotype and the intron 10 variant sits on the H1D subhaplotype. Interestingly, alternative splicing of exon 10 leads to 3R or 4R versions of MAPT encoded protein tau, with 4R being the prominent form in PSP. Additional studies will be needed to fully assess the functional impact of the MAPT intronic variants identified.

**Authors:** *J. GARTLON*¹, S. KRAUSE², Z.-Z. LI², P. MCCracken², A. GIBSON³, W. TRIGG², A. KOYAMA¹;

¹Neurosci. and Gen. Med. Product Creation Unit, ²Biomarkers and Personalized Med. Core Function Unit, Eisai Inc., Andover, MA; ³GE Healthcare Ltd., Amersham, United Kingdom

**Abstract:** Translocator protein (18 kDa) (TSPO) is expressed in the outer mitochondrial membrane of steroid-synthesising cells and may serve as a marker of microglial activation in neurodegenerative diseases (Rupprecht et al., Nature Reviews Drug Discovery, 9, 971-988, 2010). [18F]GE-180 is a TSPO-specific radiotracer developed by GE Healthcare (UK) for use in positron emission tomography (PET) imaging. The aim of this study was to measure age-related changes in [18F]GE-180 uptake in the rTg4510 transgenic mouse model of tauopathy using ex-vivo autoradiography and in-vivo PET imaging. [18F]GE-180 was synthesised on a microfluidic platform using a modified method. Autoradiography was performed on coronal sections prepared from brains of female rTg4510 mice aged 2, 8 and 14 months (m) and wild-type mice aged 2 m, using 5 MBq [18F]GE-180. For PET imaging, 15 min static scans were acquired 20 min post-injection of 10-15 MBq [18F]GE-180 to evaluate tracer uptake in the frontal, occipital and temporal cortex, hippocampus, striatum and cerebellum in 4, 9 and 12 m female rTg4510 (n = 8-9) and wild-type mice (n = 5). Regions of interest (ROI) were defined using a mouse atlas realigned to the CT image. Activity in each ROI was reported as standard uptake volume (SUV). Ex-vivo autoradiography revealed a progressive, age-related increase in signal in the cortex and hippocampus of rTg4510 mice (aged 2 - 14 m) which was blocked by 36 µM of unlabelled GE-180. PET imaging with [18F]GE-180 revealed similar age-related increases in [18F]GE-180 uptake in the cortex, striatum and hippocampus in rTg4510 mice compared to wild-type mice of the same age. For example, in the frontal cortex the SUV was increased in transgenic vs wild-type mice by 39 % at 4 m (P<0.01) and by 69 % at 12 m (P<0.01). A trend towards age-related increases in SUV in wild-type mice was also observed. For example, in the frontal cortex the SUV increased by 42 % in wild-type mice aged 12 m vs 4 m (P<0.05). Studies are ongoing in our laboratory to measure age-related regional volume changes in rTg4510 and wild-type mice using magnetic resonance imaging (MRI). These data will be used to check for any under-estimation of SUV due to discrepancies in regional volume in transgenics vs wild-types. In summary, the TSPO-specific radiotracer [18F]GE-180 was used successfully to detect an age-related increase in TSPO binding sites in wild-type mice and to a much greater extent in rTg4510 mice, suggesting that microglial activation increases as a result of both tauopathy and, to a lesser extent, natural ageing.

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**Poster**

483. Alzheimer's Disease: Tau
Title: Sigma-1 receptor regulates Tau phosphorylation by shaping p35 turnover via myristic acid

Authors: *S.-Y. A. TSAI1; M. POKRASS2; N. KLAUER3; H. NOHARA4; T.-P. SU1;
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Abstract: Dysregulation of cyclin-dependent kinase 5 (cdk5) per relative concentrations of its activators p35 and p25 is implicated in neurodegenerative diseases. P35 has a short half-life and undergoes rapid proteasomal degradation in its membrane-bound myristoylated form. p35 is converted by calpain to p25 which, along with an extended half-life, promotes aberrant activation of cdk5 and causes abnormal hyperphosphorylation of tau, leading to the formation of neurofibrillary tangles. One challenge in treating “tauopathy” is to effectively reduce the available p35 to circumvent the formation of over-reactive cdk5/p25. The sigma-1 receptor (Sig-1R) is an endoplasmic reticulum chaperone that is implicated in neuronal survival. However, the specific role of the Sig-1R in neurodegeneration is unclear. Here we found that Sig-1Rs regulate proper tau phosphorylation and axon extension by promoting p35 turnover through the receptor’s interaction with myristic acid. In Sig-1R knockout neurons, a greater accumulation of p35 is seen, which is neither due to elevated transcription of p35 nor to disrupted calpain activity, but rather to the slower degradation of p35. In contrast, Sig-1R overexpression causes a decrease of p35. Sig-1R knockout neurons exhibit shorter axons with lower densities. Myristic acid is found here to bind Sig-1R as a nanomolar affinity agonist that causes the dissociation of Sig-1R from its cognate partner BiP. Remarkably, treatment of Sig-1R knockout neurons with exogenous myristic acid mitigates p35 accumulation, diminishes tau phosphorylation, and restores axon elongation. Our results define the involvement of Sig-1Rs in neurodegeneration and provide a mechanistic explanation that Sig-1Rs help maintain proper tau phosphorylation by potentially carrying and providing myristic acid to p35 for enhanced p35 degradation to circumvent the formation of over-reactive cdk5/p25. Our results also identify the myristic acid as an endogenous Sig-1R ligand that may represent a naturally–occurring substance for shaping neuronal systems against neurodegenerative diseases.

Disclosures: S.A. Tsai: None. M. Pokrass: None. N. Klauer: None. H. Nohara: None. T. Su: None.
Title: Aβ Amyloidosis stimulates development of tau pathologies in a new Alzheimer’s mouse model

Authors: *K. E. BRAUNSTEIN, P. C. WONG, T. LI;
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Abstract: Effective therapy for Alzheimer’s disease (AD), the most common form of dementia and a devastating illness of the elderly, remains a great-unmet need. Genetic studies of early onset familial AD have fueled the notion that abnormal accumulation of A in the brain would trigger the aggregation of tau, leading to synaptic dysfunction, neurodegeneration and dementia. However, the molecular mechanism underlying such an “amyloid cascade” hypothesis remains ill defined. To determine whether the amyloid burden could accelerate tauopathy-dependent neurodegeneration, we now develop a new mouse model that express the human tau fragment containing the four repeat microtubule binding domain of tau. Consistent with the finding that expression of wild type human full-length tau in mice did not develop tau tangle, neither tau tangle nor neuronal loss is observed in these tau mice. However, when our tau mice are crossbred with a model of Aβ amyloidosis (APP$$^{sw};$$PS1E9 mice) to generate mice (Tau312-AP mice) that develop Aβ amyloidosis in the presence of this tau fragment, AD-like tau pathologies and tauopathy-dependent loss of neurons are observed. Furthermore, we show that the human tau fragment converts endogenous tau to form neurofibrillary tangles. These results strongly support the idea that Aβ amyloidosis plays a central role in stimulating the tauopathy-dependent neuronal loss in the pathogenesis of AD. Importantly, such a model of AD will be instrumental for further clarifying the molecular mechanism whereby Aβ impacts on tauopathy-dependent neurodegeneration and for evaluation of Aβ- and tauopathy-dependent loss of neurons as the principal outcome measure in preclinical testing of mechanism-based therapies.
Disclosures:  K.E. Braunstein: None. P.C. Wong: None. T. Li: None.

Poster

483. Alzheimer's Disease: Tau

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#/Poster#: 483.05/B103

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: UO1NS086659-01

P30AG13846

Dept of Veterans Affairs

Title: The distinctive TDP-43 pathology of CTE is accelerated in areas of traumatic axonal injury and co-aggregates with tau

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Abstract: CTE is a tauopathy associated with brain trauma, characterized by the widespread distribution of neurofibrillary tangle (NFTs), thorn-shaped astrocytes and other hyperphosphorylated tau deposits in the brain. The pathological severity of CTE can be divided into 4 stages (McKee et al 2013). Abnormal TDP-43 pathology is found in most cases of CTE and increases in parallel with tau pathology. TDP-43 pathology consists of abnormally rounded, dotlike structures in neuronal and glial processes and inclusions in astrocytes and neurons that partially co-localize with p-tau deposits. We examined over 150 cases of CTE in the VA-BU-SLI brain bank and found TDP-43 immunopositive neurites in the subcortical white matter and fornix, areas prone to traumatic axonal injury, in CTE Stage I. In CTE Stage II, TDP-43 dotlike, rounded structures and glial inclusions were found in the subcortical white matter, brainstem, medial temporal lobe, and retina often in a subpial, periventricular or perivascular distribution. Stage III CTE cases contained TDP-43 immunoreactive deposits in neurons and astrocytes in the cerebral cortex, medial temporal lobe, brainstem, retina and white matter, and Stage IV cases displayed TDP-43 positive rounded and threadlike neurites and glial structures, astrocytic and neuronal inclusions in cerebral cortex, medial temporal lobe, diencephalon, basal ganglia, brainstem, retina, spinal cord and white matter. In cases with the most severe TDP-43 deposition, dense accumulations of TDP-43 were found in layers II and III of the neocortex and in the
dentate fascia of the hippocampus, a distribution pattern that overlaps with the distribution of TDP-43 found in frontotemporal lobar degeneration with TDP-43 (FTLD-TDP), although the morphology of the deposits was distinctive. The morphology and distribution of TDP-43 appears to be unique in CTE, and can be distinguished from the TDP-43 pathology of Alzheimer’s disease, normal aging and FTLD-TDP. TDP-43 accumulation in CTE appears to be accelerated in regions of traumatic axonal injury and in regions of p-tau deposition. Future studies will help determine whether the distinctive rounded neuritic and astrocytic TDP-43 pathology can be used as a tool in the pathological diagnosis of CTE.


Poster

483. Alzheimer's Disease: Tau

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 483.06/B104

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant F32NS089281

Tau Consortium

JPB Foundation

Cure Alzheimer's Fund

Title: Sleep disturbance progression in P301S tau transgenic mice

Authors: *J. HOLTH, G. ROBINSON, D. M. HOLTZMAN;
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Abstract: Tauopathies are a class of neurodegenerative disorders associated with abnormal, pathological aggregation of tau protein. This classification includes diseases such as Progressive supranuclear palsy (PSP), Corticobasal degeneration (CBD), some forms of Frontotemporal Dementia (FTD), and Alzheimer’s disease (AD). In addition to neurodegeneration, tauopathies are associated with an increased prevalence of sleep disturbances including insomnia, excessive daytime sleepiness, and rapid eye movement (REM) sleep behavior disorder. Up to 76% of FTD and 66% of AD patients exhibit sleep disturbances of some kind and a high percentage of CBD and PSP patients suffer from insomnia. These sleep disorders are also a major cause of
Sleep is an important biological process that facilitates learning and memory as well as metabolic homeostasis of proteins within the brain. Despite the importance of sleep and the high prevalence of sleep disturbances in tauopathies, little is known about the role of tau pathology in sleep disruption. Studies in a mouse model of AD expressing both tau and amyloid pathology have shown that sleep deficits occur when tau pathology is present but before amyloid pathology is abundant. In this study we utilized P301S human tau transgenic mice to analyze if tau pathology development and progression alone is sufficient to alter sleep. P301S mice develop progressive tau pathology with the onset of tau aggregation around 5 months of age and neuronal loss at 8 months. Using EEG/EMG sleep analysis at 6, 9 and 11 months of age, we show that P301S mice with significant tau aggregation and pathology have sleep deficits that worsen with age. Compared to wild type, P301S mice have decreased REM sleep at 9 months of age and decreased REM and NREM sleep as well as increased wakefulness at 11 months. No changes are observed at 6 months of age. Furthermore, compared to wild type, P301S mice have increased cortical EEG power in delta and theta frequencies at 9 months of age but significantly decreased EEG power at 11 months. The REM sleep EEG power distribution at 11 months of age is also altered. These results suggest that tau pathology disease progression is sufficient to alter sleep in P301S mice. Understanding this interaction between sleep disturbances and tau pathology could lead to better diagnostics and treatments for tauopathy-associated sleep disorders, decreases in institutionalization, and possibly provide a strategy for slowing disease progression itself.

Disclosures: J. Holth: None. G. Robinson: 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.; AstraZeneca, Eli Lilly, C2N Diagnostics. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Cure Alzheimer's fund, JPB Foundation, Tau Consortium. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); C2N Diagnostics LLC. F. Consulting Fees (e.g., advisory boards); AstraZeneca, Genentec, Eli Lilly, Neurophage, C2N Diagnostics.

Poster

483. Alzheimer's Disease: Tau

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 483.07/B105

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Grant-in-Aid for Young Scientists (Start-up) 26893337
Title: Vascular β-amyloid and tau: bidirectional influence in APP and MAPT bigenic mice

Authors: *S. SAITO1,2, M. IHARA3, Y. OKAMOTO4, Y. HATTORI5, Y. YAMAMOTO2, A. KITAMURA1, R. TAKAHASHI1;
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Abstract: Alzheimer’s disease (AD) is characterized by parenchymal β-amyloid (Aβ) plaques, cerebral amyloid angiopathy (CAA), and neurofibrillary tau tangles. Several studies using amyloid precursor protein (APP) and microtubule-associated protein tau (MAPT) bigenic mice showed enhanced neurofibrillary degeneration without any alterations in Aβ plaques, indicating that tau is downstream of Aβ. One report has shown greater Aβ deposition in double transgenic APP/tau mice than in single APP transgenic mice although interaction between Aβ and tau remains to be clarified. AD is attributed to both excess production and impaired clearance of Aβ but the latter may be more crucial in sporadic AD. Mice overexpressing the human APP gene with Swedish-Dutch-Iowa mutations (Tg-SwDI mice) model CAA in that Aβ clearance is impaired. Here, we have developed a double transgenic mouse by crossing Tg-SwDI mice with Tg-P301S mice carrying the human MAPT gene with P301S mutation to investigate the role of CAA in tau metabolism. Immunohistochemical staining showed that the amount of total tau, 4 repeat tau, and hyperphosphorylated tau was more abundant in the double Tg mice than Tg-P301S mice. The double Tg mice showed most prominent tau accumulation in the subiculum where Aβ deposits first appear in Tg-SwDI mice, while they exhibited significantly increased Aβ deposition in the dentate gyrus where tau accumulation is marked in Tg-P301S mice. The Aβ deposits were mainly observed in the capillaries and composed of Aβ40. The double Tg mice showed more pronounced astrogliosis, microgliosis, and neuronal loss than wild type mice and single Tg mice, the greatest reduction of cerebral blood flow in the cortex as estimated with laser speckle flowmetry, and significantly impaired spatial reference memory as evaluated in the Barnes maze test. These findings indicate bidirectional influence between vascular Aβ and tau, suggesting a positive feedforward loop of toxic protein accumulation. Such vicious cycle may explain one of the pathomechanisms of sporadic AD.


Poster

483. Alzheimer’s Disease: Tau

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM
Title: BRI3 allelic variants are associated with cerebrospinal fluid levels of phosphorylated-tau in Alzheimer’s disease

Authors: *K. D. DETERS¹, K. NHO², S. L. RISACHER², S. KIM², R. VIDAL³, A. J. SAYKIN²;
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Abstract: Background: Alzheimer’s disease (AD) is a progressive neurodegenerative disorder characterized neuropathologically by amyloid-beta (Aβ) plaques and neurofibrillary tangles comprised of hyperphosphorylated tau protein. Aβ is formed after the sequential cleavage of the amyloid precursor protein (APP) by β- and γ-secretase. BRI3 is predominantly expressed in brain and is able to bind APP and inhibit Aβ production by blocking the α- and β-secretase binding sites. Because of this interaction, we hypothesized that BRI3 may be involved in AD pathogenesis. Therefore, we aimed to identify novel genetic associations between BRI3 and cerebrospinal fluid (CSF) analytes (Aβ, total-tau, phosphorylated-tau (p-tau)) in participants from the Alzheimer’s Disease Neuroimaging Initiative (ADNI). Methods: Whole-genome sequencing data of 584 white, non-Hispanic participants from the ADNI cohort were analyzed to identify common variants (minor allele frequency > 5%) within BRI3, resulting in 160 single nucleotide polymorphisms (SNPs) after standard quality control steps. To investigate the association of BRI3 variants with CSF analytes, both gene- and SNP-based linear regression methods were utilized. For the gene-based test, an empirical p-value was calculated using permutation testing. To correct for multiple comparisons for the SNP-based tests, a Bonferroni correction (correction factor derived from the number of linkage disequilibrium (LD) blocks in BRI3) was applied. Independent and significant SNPs were analyzed simultaneously as a multi-marker test using linear regression methods. Age, gender, and APOE ε4 status were used as covariates for all analyses. Results: Gene-based analysis of BRI3 was only significantly associated with CSF p-tau (p = 0.013). After SNP-based analysis and Bonferroni correction (p =0.05 / 15 LD blocks =0.0033), the minor allele of four SNPs within the BRI3 gene were significantly associated with decreased levels of CSF p-tau (rs1561324, p = 0.0012; rs934820, p = 0.00132; rs73992921, p = 0.00136; rs3806537, p = 0.00236). rs934820 was in LD with other
SNPs and was excluded from the subsequent multi-marker test. Using the remaining three independent SNPs for multi-marker analysis, a strong association with CSF p-tau was observed (GGC, p = 0.0001). Conclusions: The BRI3 gene is a member of the BRI gene family, with mutations in BRI2 causing diseases similar to AD. To our knowledge, this is the first study to investigate BRI3 with AD endophenotypes reflecting tau and Aβ. These results show that BRI3 is associated with CSF p-tau, a well-established biomarker of AD. Further study and replication is needed to fully understand the role of BRI3 in AD.


Poster

483. Alzheimer's Disease: Tau

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 483.09/B107

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: MEXT Grant 15K15272

MEXT Grant 26293167

Title: Tau phosphorylation increases in Alzheimer's disease mice model with diabetes associated with altered expressions of the genes involved in inflammation, insulin signaling, glucose-energy metabolism and protein quality control


Dept of geriatric medicine, Dept of psychiatry, Dept of clinical gene therapy, Osaka Univ. Grad Sch. Med., Osaka, Japan; Natl. center for neurology and psychiatry, Kodaira, Japan; Niigata Univ., Niigata, Japan; Tokyo metropolitan institute of gerontology, Tokyo, Japan

Abstract: [Background] Emerging evidence suggests that diabetes affects cognitive function and increases the incidence of dementia. We previously reported APP+ob/ob mice showed cognitive dysfunction. However, the mechanisms by which diabetes modifies cognitive function still remains unclear. In comparison with the characteristics of AD brain structure and cognition, diabetes seems to affect cognitive function through not only simple AD pathological feature-dependent mechanisms, but also independent mechanisms (Sato et al. Frontiers in
Endocrinology. doi: 10.3389/fendo.2014.00143). The aim of this study is to explore the mechanisms by which diabetes modifies AD using AD mice models with diabetes. [Methods] We previously reported that APP+ob/ob mice showed more severe cognitive dysfunction than APP transgenic mice (Takeda, Sato et al. PNAS, 13, 107, 7036-41, 2010). We performed western blotting using antibodies for tau phosphorylated at various sites. We isolated RNA from brains of APP mice with high fat diet (HFD) and APP+ob/ob mice and performed microarray analysis and validation by real-time PCR. [Results] APP+ob/ob mice showed aberrant tau phosphorylation at T181, T205, T212 and S422. APP+ob/ob mice also showed altered expressions of the genes involved in inflammation, insulin signaling, glucose-energy metabolism and protein quality control. Tgfbr2, a receptor of an anti-inflammatory cytokine, TGFβ, was up-regulated in HFD-fed APP mice and APP+ob/ob mice. Interleukine-1β (IL1β), a pro-inflammatory cytokine, was up-regulated in APP+ob/ob mice. The expression of insulin receptor substrate 2 (Irs2) was increased significantly in the APP+ob/ob mice. Prkab1, non-catalytic subunit of AMP-activated protein kinase (AMPK), was up-regulated in HFD-fed APP mice and in APP+ob/ob mice. Ubiquitin ligase encoding Ube3A was increased in HFD-fed APP mice and in APP+ob/ob mice. [Conclusions] Tau phosphorylation increases in Alzheimer disease mice model with diabetes associated with altered expressions of the genes involved in inflammation, insulin signaling, glucose-energy metabolism and protein quality control. Further studies are required to determine the precise contribution of these genes in the pathogenesis in AD and diabetes. And taking together, this aberrant phosphorylation of tau in brain degeneration during AD and diabetes might be induced via cell autonomous and non-cell autonomous mechanisms.


Poster

483. Alzheimer's Disease: Tau

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 483.10/B108

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NHMRC

Title: Increasing bioavailable copper targets Tau pathology via the phosphatase PP2A in a mouse model of Alzheimer’s disease
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Abstract: Alzheimer’s disease is the worldwide leading cause of dementia and affects up to 50% of the ageing population above 85. The pathogenesis of the disease remains to be fully elucidated and the current lack of therapies reflects this. Recently, intense research suggests that metals may play crucial roles in the pathogenic process. Indeed, treatment with copper delivery compounds has yielded therapeutic benefits. However, the precise mechanism of action is still being investigated. In this study, AD transgenic (APP/PS1) mice were treated with the intracellular copper delivery compound, CuII(GTSM). Treatment rescued spatial memory deficits in the Morris water maze. Protein analysis indicated that treatment reduced the levels of phosphorylated forms of tau, consistent with previous reports. There was also a reduction in the levels of pathological aggregated Tau. Treatment additionally increased the level of all subunits of the serine/threonine phosphatase protein phosphatase 2A (PP2A), responsible for dephosphorylating Tau. These studies suggest that increasing bioavailable intracellular copper can increase levels of PP2A, which result in a reduction in pathological aggregated Tau, possibly via a reduction on phosphorylated Tau. As it has been shown that PP2A levels and activity are reduced in AD brains, this study shows that targeting this pathway is crucial in reversing pathology and further validates increasing bioavailable intracellular copper as an effective therapy for AD. Furthermore, the treatment did not alter amyloid-β levels effectively placing amyloid-β above Tau in the toxicity cascade and validates Tau as a potential therapeutic target.

Title: α-Synuclein modulates amyloid-β oligomer toxicity, tau phosphorylation, and ectopic cell cycle re-entry in neurons

Authors: *S. S. THOMAS, G. S. BLOOM;
Univ. of Virginia, Charlottesville, VA

Abstract: α-Synuclein aggregates, in the form of Lewy body inclusions, and tau neurofibrillary tangles, are often co-detected post-mortem in a spectrum of neurodegenerative diseases, including Alzheimer’s and Parkinson’s. A large body of evidence supports the hypothesis that α-synuclein and tau share a synergistic and deleterious relationship. However it is unknown why, or if, expression of both proteins is required to initiate disease progression and death in neurons. Importantly, no studies conducted in Alzheimer’s models have carefully investigated the role of endogenous α-synuclein in disease progression. Using mouse-derived cortical neuron cultures treated with toxic amyloid-β oligomers (AβOs) to model Alzheimer’s we tested whether tau-dependent disease phenotypes are affected by α-synuclein reduction or overexpression. We found that AβOs increase the expression of α-synuclein, but only in the presence of endogenous tau. Furthermore, α-synuclein expression is necessary for the AβO-stimulated ectopic neuronal cell cycle re-entry (CCR) that accounts for substantial neuron death in Alzheimer’s. Phosphorylation of tau at serine 409, an indicator of Alzheimer’s pathogenesis and a requirement for CCR, is also attenuated by reduction of α-synuclein. Interestingly, overexpression of mutant, but not wild type (WT) α-synuclein in WT neurons is sufficient to increase neuronal CCR and elevate tau phosphorylation at sites required for CCR independently of AβOs. In contrast, overexpression of mutant α-synuclein does not induce CCR in neurons derived from tau knockout mice, even in the presence of extracellular AβOs. Taken together, these results support the hypothesis that α-synuclein and tau interdependently promote neuronal dysfunction, particularly CCR. Targeting this interaction potentially represents a novel therapeutic approach for the treatment of Alzheimer’s, Parkinson’s, and other neurodegenerative diseases that involve synergism between α-synuclein and tau.

Disclosures: S.S. Thomas: None. G.S. Bloom: None.

Poster

483. Alzheimer’s Disease: Tau

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 483.12/B110
Title: Early tau pathology within cholinergic nucleus basalis neurons coincides with neurotrophic gene dysregulation during the progression of Alzheimer’s disease

Authors: *C. T. TIERNAN*¹, S. M. WARD¹, A. L. GUILLOZET-BOGAARTS², N. M. KANAAN¹, B. HE³, S. D. GINSBERG⁴, E. J. MUFSON³, L. I. BINDER¹, S. E. COUNTS¹; ¹Translational Sci. and Mol. Med., Michigan State Univ., Grand Rapids, MI; ²Allen Inst. for Brain Sci., Seattle, WA; ³Div. of Neurosci., Barrow Neurolog. Inst., Phoenix, AZ; ⁴Ctr. for Dementia Res., Nathan Kline Inst., Orangeburg, NY; ⁵Dept. of Psychiatry, ⁶Dept. of Physiol. and Neurosci., NYU Langone Sch. of Med., New York, NY

Abstract: Cholinergic nucleus basalis (NB) neurons provide the major cholinergic innervation to the cortical mantle and are exquisitely prone to tau pathology and neurofibrillary tangle (NFT) formation during the progression of Alzheimer’s disease (AD). However, the molecular and cellular relationships between the evolution of tau pathological processes and NB cell survival remain unknown. NB neurons require the neurotrophin nerve growth factor (NGF) and its receptors, TrkA and p75(NTR), for their maintenance and survival. Therefore, we profiled NGF pathway genes in NB neurons immunostained for the pS422 tau epitope, which identifies an early phosphorylation event preceding tau C-terminal truncation at D421, or dual-labeled for pS422 and TauC3, a later stage tau neo-epitope revealed by the C-terminal truncation event. These phenotypically distinct NB neurons (n = 40-80/phenotype) were individually microaspirated from tissue sections harvested postmortem from Rush Religious Orders Study participants who died with an antemortem clinical diagnosis of no cognitive impairment (NCI), mild cognitive impairment (MCI, a putative prodromal AD stage), or mild/moderate AD (n = 8-10/group). Each neuron was processed for RNA amplification and custom-designed microarray analysis. Quantitative evaluation revealed a significant ~50% downregulation in mRNA encoding the cognate NGF receptor TrkA in pS422-labeled compared to pS422-negative NB neurons. In addition, the cognate brain-derived neurotrophic factor and neurotrophin-3 receptors TrkB and TrkC, as well as the Trk-mediated downstream pro-survival kinase Akt, were downregulated ~40-60% in pS422+ cells. These transcripts were not further downregulated in pS422/TauC3 dual-labeled NB neurons. By contrast, levels of the pan-neurotrophin receptor p75(NTR) were downregulated by ~35% in dual-labeled neurons. Transcripts encoding choline acetyltransferase (the synthetic enzyme for acetylcholine), β-amyloid precursor protein (APP), APP family members, and regulators of APP metabolism were not differentially regulated across the tau phenotypic cell groups. Notably, we found that gene expression patterns for each cell phenotype did not differ with clinical diagnosis, suggesting that differential gene regulation...
within NB neurons is related to their pathological status and not the cognitive status of the individual. Taken together, these cell-type specific microarray data suggest that a dysregulation of neurotrophic cell survival signaling is an early pathogenic mechanism associated with NFT formation in vulnerable cholinergic NB neurons in AD, which may be amenable to therapeutic intervention.


Poster

483. Alzheimer’s Disease: Tau

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 483.13/B111

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: OP VaVPI

- REGPOT ICRC-ERA-Human Bridge
- ICRC Human Bridge-Talent Incubator

Title: Disruption of 3R/4R tau ratio impairs APP axonal transport in human Embryonic Stem Cells (hESC) derived neurons

Authors: *V. LACOVICH1, M. ALLOATTI2, T. FALZONE2, S. ESPINDOLA3, M. E. AVAL63, M. CARNA1, G. FORTE1, G. B. STOKIN1;
1Intl. Clin. Res. Ctr. (FNUSA-ICRC, Brno, Czech Republic; 2Inst. de Biologia Celular y Neurociencias (UBA-CONICET), Facultad de Medicina, Univ. de Buenos Aires, Buenos Aires, Argentina; 3Inst. de Ingeniería genética y Biología Mol. (INGEBI-CONICET), Buenos Aires, Argentina

Abstract: Alzheimer’s disease is characterized by the abnormal abundance of two hallmark proteins, APP and tau. In the last two decades several studies have tried to propose a molecular cascade of events leading to the pathology. One of the approaches to study the mechanisms leading to disease is to use transgenic mice bearing mutations that have been described in familiar cases of AD. The triple-transgenic model (3xTg-AD; PS1M146V, APPSWE and tauP301L) developed an age-related and progressive neuropathological phenotype that included both plaques and tangles, providing evidence of amyloid cascade hypothesis, by which Aβ
deposits precede tau alterations. However it was later shown that if tau is knocked out, there are no amyloid related changes, thus leaving an open question about how APP and tau are interlinked. To test this hypothesis further we have produced changes in the tau isoforms by using a spliceosome-mediated RNA trans-splicing system (SMaRT). We induced tau imbalance in mature hESCs derived neurons using pseudolentiviral particles, leading the expression of tau with 3 or 4 microtubule-binding repeats, thus disrupting the normal 3R/4R tau ratio. A cherry pseudolentiviral control was used to assess the transduction rate. Five days after transduction, transfections with APP-YFP were performed 24 hours prior to imaging. After identifying the neuron’s axons, we recorded the axonal transport of APP-YFP in 30s movies. Kymographs generated from the recordings were analysed and mobility parameters of single particle trajectories such as run-length, average and segmental velocities were calculated. We confirmed the 3R/4R tau imbalance by measuring tau RNA and protein levels. Cell cultures with predominant 3R or 4R tau expression exhibited impaired axonal transport of APP-YFP compared with control. More specifically, there was a significant reduction of average velocity and segmental velocity distribution. Intriguingly analyses of all parameters of axonal transport indicated consistently significant impairments in anterograde axonal transport with milder effect on retrograde axonal transport. Our findings indicate that changes in tau isoforms play a role in regulating APP axonal transport. Further experiments will be needed to fully characterize the relation between tau and APP.


Poster

483. Alzheimer's Disease: Tau

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 483.14/B112

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Motor speech phenotypes of frontotentorial dementia and primary progressive aphasia: a review of behavioral and brain imaging findings

Authors: *M. L. POOLE1,2, A. BRODMANN2,3, D. DARBY2,3, A. P. VOGEL1,2;
1Audiol. and Speech Pathology, Univ. of Melbourne, Parkville, Australia; 2Eastern Cognitive Disorders Clin., Melbourne, Australia; 3Dept. of Behavioural Neurosci., Florey Inst. of Neurosci. and Mental Hlth., Melbourne, Australia
Abstract: Objectives: To review the behavioral and neurological profile of motor speech impairments in frontotemporal dementia and primary progressive aphasia syndromes. Background: Frontotemporal dementia (FTD) and primary progressive aphasia (PPA) are terms used to group a number of related neurodegenerative disorders. They are united by overlapping pathologies and significant impairment to communication. They include the clinical presentations of behavioural variant FTD (bvFTD), nonfluent variant FTD (nfvFTD or nfvPPA) and semantic variant FTD (svFTD or svPPA). An atypical onset of Alzheimer’s disease, logopenic PPA (lvPPA) constitutes a further PPA variant, and many authors consider primary progressive apraxia of speech (PPAOS) to be a distinct syndrome. Motor speech impairments are common, either directly, as part of the symptom profile, or associated with concomitant motor neuron disease or parkinsonism. Method: 589 studies were identified from a search of the MEDLINE database. Abstracts were reviewed and studies included if they reported speech outcomes using quantitative or validated scales. Neuroimaging data were collated when imaging was compared to speech measures. Results: Forty-two articles were included in the review. Subjective (listener based) measures of speech were reported in 24 studies and objective (acoustic) assessments were reported in 19 studies which included participants diagnosed with bvFTD, nfvPPA, svPPA, lvPPA and PPAOS. Six studies correlated speech measures with neuroimaging findings. A range of brain/behaviour associations were reported. Speech production in people with nfvPPA was correlated with atrophy of the supplementary motor area (SMA), precentral gyrus and inferior frontal gyrus. White matter tract abnormalities connecting the SMA, pars opercularis, primary motor cortex and caudate were also linked to deficits in speech production in nfvPPA. Conclusions: Quantitative measures of speech correlate with the clinical diagnostic criteria for FTD and PPA. Motor speech impairments are common in nfvPPA, but are rare in svPPA and lvPPA, and inadequately documented in bvFTD. Several neuroanatomical regions which can be affected by these disorders are highlighted for their importance in speech production. Further, the review indicates the utility of quantifiable measures of speech as a useful clinical tool in classifying PPA subtype.

Disclosures: M.L. Poole: A. Employment/Salary (full or part-time); National Health and Medical Research Council. A. Brodmann: None. D. Darby: None. A.P. Vogel: None.

Poster

483. Alzheimer's Disease: Tau

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 483.15/C1

Topic: C.02. Alzheimer's Disease and Other Dementias
**Title:** Temperature is involved in tau exon 10 alternative splicing regulation

**Authors:** *F. PETRY;*
CHUL Reserach Ctr., Quebec, QC, Canada

**Abstract:** Tau is a microtubule-associated protein abundant in neurons. Its main function, bind and stabilize MTs, is mediated by the MT binding domain consisting of 3 or 4 repeated regions. Alternative splicing of tau exon 10 results in the presence or the absence of a MT-binding repeat, leading to the expression of tau with either four (4R-tau) or three (3R-tau) MT-binding repeats. In human brain development, 3R-tau are expressed from the embryonic stages while 4R-tau expression begins after birth. An equal amount of 3R-tau and 4R-tau is expressed in adult human brain. Alteration of the ratio is thought to be causing several neurodegenerative diseases, called tauopathies. Interestingly, a different pattern of tau isoforms during mice development was reported. Indeed, 3R-tau are only expressed during the first developmental stages and their expression disappeared in adulthood. Preliminary data on body pups temperature show that pups are hypothermic during the first developmental stages, correlating with the expression of 3R-tau isoforms expression. The purpose of this study is to analyze the regulation of exon 10 splicing and the mechanisms involved in the differential expression of 3R and 4R-tau in mice development. In a new way, we also investigate the impact of the temperature in the regulation of exon 10 splicing. We have analyzed tau and splicing factors during mouse brain development. To analyze the impact of temperature in adulthood, we starved adult mice to induce hypothermia. Finally, we used N2a cells, a cell line known to express both 3R-tau and 4R-tau, and exposed them to direct hypothermia. Our results show an increase of SR proteins promoting the inclusion of exon 10 and a decrease of those promoting exclusion during mice development, which is consistent with the profile of tau isoforms. We also observed re-expression of 3R-tau mRNA in starved mice, confirming the hypothesis that temperature regulate tau exon 10 splicing. We have also seen an increase of splicing factor promoting exon 10 exclusion. N2a cells at 32°C showed an increase of 3R-tau at mRNA and protein levels, followed by an increase of splicing factors promoting exon 10 exclusion. All together, these result indicate that temperature is involved in the regulation of the alternative splicing of tau exon 10, by changing splicing factors expression. During development, tau exon 10 splicing well correlate with splicing factor expression and hypothermia seen in the first post-natal developmental stages may also contribute to the expression of 3R-tau and its decline in adulthood. Here, we are the first to propose temperature as a key regulator of exon 10 splicing.

**Disclosures:** F. Petry: None.

**Poster**

**483. Alzheimer's Disease: Tau**

**Location:** Hall A
Title: Comparative distribution of early and late appearing epitopes of tau in tauopathies

Authors: *O. MELÉNDEZ-FERÁNDEZ, E. Y. KAO, S. WEINTRAUB, E. BIGIO, M.-M. MESULAM, C. GEULA;
Cognitive Neurol. and Alzheimer's Dis. Ctr., Northwestern Univ., Chicago, IL

Abstract: Tauopathies are characterized by aggregation of hyperphosphorylated tau in glial cells and/or neurons. Differential accumulation and distribution of abnormal tau protein, along with specific morphologic characteristics of tau inclusions, contribute to the distinct pathology observed in each tauopathy. In Alzheimer's disease (AD), different phosphorylated and truncated epitopes of tau appear early, intermediate or late in the process of tangle formation. In this study we aimed to compare the presence and distribution of early versus late tau epitopes across AD, progressive supranuclear palsy (PSP), corticobasal degeneration (CBD) and Pick's disease (PiD). We employed the novel antibody tau oligomeric complex 1 (TOC1), which identifies oligomeric non-fibrillar forms of tau and appears early in the process of tangle formation, and MN423, a truncated epitope of tau which appears late in the process of tangle formation in AD. The AT8 antibody, which recognizes tau phosphorylated at Ser202/Thr205 and is routinely used for pathologic diagnosis of tauopathies, was employed to select the brain regions most affected in each tauopathy for analysis. TOC1 immunoreactivity was robustly distributed in neurites and intracellular inclusions across all tauopathies. TOC1 positivity was detected in pre-tangles, some tangles, dystrophic neurites and a dense population of neuropil threads in AD; in neuronal and glial cytoplasm, neuropil threads and astrocytic plaques in CBD; in neuronal and glial cytoplasm, tangles, and neurites in PSP, and in Pick bodies, neurites and glial cells in PiD. The TOC1 antibody revealed tau pathology distribution similar to AT8 stained structures, but with more neurites stained. MN423 was present in a large population of tangles and neurites in AD, and in a scattered population of glial cells and neurites in PiD. Only sparsely distributed neurites and occasional intracytoplasmic staining of cells with glial morphology were MN423 immunoreactive in PSP and CBD. These findings are consistent with previous reports pointing to both similarities and distinctions between tauopathies in relation to epitopes of tau present in each. Specifically, while the initial stages of tau abnormalities appear to be similar in all tauopathies, the progression of pathology seems to follow distinct paths. Distinguishing the
specific epitopes found in each tauopathy can contribute to a better understanding of pathology progression, allowing imaging probes to be tailored to identify \textit{in vivo} tau accumulation specific to each tauopathy. This is likely to lead to therapeutic approaches targeting distinct pathways identified in the progression of tau pathology.


Poster

483. Alzheimer's Disease: Tau

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#/Poster#: 483.17/C3

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: BrightFocus Foundation

Cure Alzheimer Foundation

NINDS

NIEHS

Title: Association of tau with the stress granule RNA-binding protein TIA1 regulates tau pathology and neurodegeneration

Authors: *D. APICCO, T. VANDERWEYDE, P. ASH, K. YOUMANS-KIDDER, A. FRAME, B. WOLOZIN,
Pharmacol. & Exptl. Therapeut., Boston Univ. Sch. of Med., Boston, MA

Abstract: Increasing evidence links neurological disease processes to dysfunction of RNA binding proteins (RBPs). RBPs regulate all facets of RNA metabolism and have particularly crucial roles in neuronal cells that require extensive splicing, transport, and local translation of specialized transcripts. RBPs also possess conserved hydrophobic, prion-like domains that facilitate their reversible aggregation in stress granules (SGs), which are RNA-protein complexes that form transiently in response to stress-induced translational arrest. Disease-linked mutations in RBPs increase the tendency of these proteins to aggregate in SGs, leading to the formation of stable, pathological SGs. Primary nucleating SG proteins, such as T-cell intracellular antigen 1 (TIA1), co-localize with neuropathology in brain tissue of subjects with Amyotrophic Lateral Sclerosis (ALS), Alzheimer’s Disease (AD), and frontotemporal dementia (FTD). Here, we
report a novel role for microtubule associated protein tau in SG biology, and demonstrate that the interaction of tau with TIA1 promotes tau pathology and neurodegeneration. Tau and TIA1 co-immunoprecipitate and progressively co-aggregate in various mouse models of tauopathy. Overexpression of tau accelerates SG formation and increases SG size. Conversely, genetic deletion of tau reduces SG formation and abrogates the binding of TIA1 to various proteins in its core proteome. Further, the association of tau with TIA1 regulates the pathophysiology of tau. Overexpression of TIA1 stabilizes tau in granules, induces tau misfolding, and stimulates neurodegeneration. Importantly, TIA1 is required for the neurotoxic effect of the disease-linked P301L tau mutation since TIA1 knockout rescues dendritic shortening and caspase activation induced by P301L tau. Pharmacological agents that increase SG formation (i.e. puromycin or salubrinal) potentiate TIA1-induced neurotoxicity while compounds that inhibit SG formation (i.e. cycloheximide) attenuate tau pathology and neurodegeneration. These results point to the translational stress response as a novel tau regulatory pathway and highlight new therapeutic strategies for the treatment of tauopathies such as AD and FTD.


Poster

483. Alzheimer's Disease: Tau

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 483.18/C4

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: MRC

Title: The increase of L-type calcium channel density produced by overexpression of human tau might underlie augmentation of the afterhyperpolarization in rat hippocampal neurons

Authors: *T. W. CHURCH*¹, E. M. RANDALL¹, J. R. MONTOMERY², J. T. BROWN³, N. V. MARRION¹;

¹Univ. of Bristol, Bristol, United Kingdom; ²Takeda Cambridge Ltd, Cambridge, United Kingdom; ³Univ. of Exeter, Exeter, United Kingdom

Abstract: Tauopathies, including Alzheimer’s disease and frontotemporal dementia, are neurodegenerative diseases characterised by cognitive impairment. Reduced action potential firing, arising from augmented afterhyperpolarizations (AHPs) has been postulated to contribute to these intellectual impairments. A large component of AHPs in hippocampal neurons is
calcium (Ca²⁺) dependent, with L-type channels implicated in being the source of Ca²⁺. Overexpression of human tau isoform 4R0N augmented the currents underlying AHPs in rat CA1 hippocampal pyramidal neurons within an organotypic slice preparation. The current underlying the medium AHP (ImAHP) increased by 111%, while the current underlying the slow AHP (IsAHP) was augmented by 71%, when compared with currents recorded from CA1 neurons expressing eGFP alone. Expression of L-type CaV1.2 or CaV1.3 with αδ1 and β2A or 3 subunits in TA201 cells, resulted in voltage-dependent macroscopic currents recorded under whole-cell voltage-clamp conditions. Coexpression of human tau isoforms 4R0N or 4R2N resulted in larger macroscopic currents only when Ca²⁺ channel subunits were expressed with β3. CaV1.2-mediated current was increased 68% by coexpression of 4R0N, and 54% by coexpression of 4R2N. CaV1.3-mediated current was increased 23% by coexpression of human tau 4R2N. Neither human tau isoform had effect on macroscopic CaV1.2 or 1.3 current when expressed with β2A. Conversely, coexpression of human tau isoform 4R1N had no effect on expressed L-type Ca current regardless of the pore-forming or β subunits. Increased macroscopic current did not result from a shift of voltage dependence or sensitivity to voltage. The sensitivity of the ImAHP and IsAHP to L-type Ca channel inhibitors was investigated. The dihydropyridine antagonist nimodipine (10 µM) inhibited the ImAHP by 51% and the IsAHP by 53% in cells expressing eGFP alone. Augmented ImAHP and IsAHP in cells overexpressing human tau 4R0N were inhibited by 69% and 68% respectively by nimodipine. The increase in the amount of current in both AHP components sensitive to inhibition by nimodipine was mirrored by isradipine. These data suggest that two isoforms of human tau (4R0N and 4R2N) can increase the number of functional L-type Ca²⁺ channels, in a β subunit-dependent manner. The increase in L-type Ca²⁺ channel number augmented the amplitude of the Ca²⁺-dependent components of the medium and slow AHPs in CA1 hippocampal neurons. The enhanced AHP increased spike accommodation, which may contribute to cognitive impairment in certain tauopathies.


Poster

483. Alzheimer's Disease: Tau

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 483.19/C5

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Mouse strain differences and tau pathology status contribute to variability in adeno-associated viral vector-mediated shRNA knockdown
**Authors:** *T. A. DAY*¹, Z. YANG¹, D. L. CZILLI¹, J. M. WOLAK², Z. AHMED², S. BOSE², M. J. O’NEILL², P. C. MAY¹;
¹Neurosci Discov Res., Eli Lilly & Co./Llc, Indianapolis, IN; ²Neurosci Discov Res., Eli Lilly & Co./Llc, Windlesham, Surrey, United Kingdom

**Abstract:** Background: Adeno-associated viral (AAV) vector gene delivery is a popular choice for CNS research due to its reported safety, efficacy, specificity and stability. Pilot studies in male C57BL/6 mice showed robust and reproducible AAV9 transduction of either GSK3β shRNA or caspase-6 cDNA in the hippocampus. In contrast, two separate studies in rTg4510 mice showed reduced expression and significant variability in AAV9-GSK3β shRNA knockdown and AAV9-Caspase-6 expression. A head-to-head comparison study in male C57BL/6 mice and female rTg4510 homozygotes and wild-type littermates was conducted to identify a potential strain-related AAV9-shRNA transduction difference.

**Methods:** AAV9-GSK3β shRNA-eGFP or AAV9-scrambled shRNA-eGFP was delivered unilaterally to four month old male C57BL/6 mice and three month old female rTg4510 homozygotes and wild-type littermates via a single stereotaxic CA1 hippocampal injection (1.1 x 10¹⁰ GCs). Six weeks post-transduction, animals were euthanized and hippocampal brain tissue was processed for quantitation of GSK3β gene knockdown and eGFP spread via qRT-PCR.

**Results:** At six weeks post-transduction, GFP expression was present within the hippocampus of all AAV9-transduced mice and correlated with GSK3β gene knockdown. GSK3β gene knockdown variability was observed in all groups, but was most pronounced in female rTg4510 mice. The greatest amount of GSK3β gene knockdown (~60%) was observed in male C57BL/6 mice, while the lowest amount of GSK3β gene knockdown (~15%) was observed in female rTg4510 homozygote transgenic mice. The female rTg4510 wild-type littermates showed intermediate gene knockdown. Conclusions: Robust GFP expression correlated with gene-targeted knockdown in male C57BL/6 mice at six weeks post-transduction with AAV9-GSK3β shRNA-eGFP. However, AAV9-shRNA gene-targeted knockdown and eGFP expression was reduced and more variable in the rTg4510 tau transgenic mouse model, indicating a strain-related AAV9-shRNA transduction difference that was exacerbated by tau pathology status.

**Disclosures:** T.A. Day: A. Employment/Salary (full or part-time): Eli Lilly & Co.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Eli Lilly & Co. Z. Yang: A. Employment/Salary (full or part-time); Eli Lilly & Co.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Eli Lilly & Co. D.L. Czilli: A. Employment/Salary (full or part-time); Eli Lilly & Co.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Eli Lilly & Co. J.M. Wolak: A. Employment/Salary (full or part-time); Eli Lilly & Co.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Eli Lilly & Co. Z. Ahmed: A. Employment/Salary (full or part-time); Eli Lilly & Co.. E. Ownership Interest
Effects of thyroid hormone upon insulin signaling pathway and tau protein in the hippocampus of diabetic rats

**Title:** Effects of thyroid hormone upon insulin signaling pathway and tau protein in the hippocampus of diabetic rats

**Authors:** *F. P. ALMEIDA, A. PANVELOSKI-COSTA, S. S. TEIXEIRA, M. NUNES, A. S. TORRAO;
Inst. of Biomed. Sci., Sao Paulo, Brazil

**Abstract:** Many studies have shown that insulin affects several brain functions including cognition and memory and have pointed out that insulin deficiency and/or resistance of the brain may be related to Alzheimer's disease (AD), suggesting a relationship between AD and diabetes mellitus (DM). In addition, several studies also suggest a modulation of thyroid hormones (TH) on insulin signaling and in the function of nervous system, showing that its deficiency or excess are involved in neurological symptoms. However, little is known about the effects of TH in the adult nervous system. The aim of this study was to evaluate the effects of TH (triiodothyronine-T3) treatment upon proteins related to insulin signaling pathway and neurodegeneration markers in the brain of diabetic rats. Adult male Wistar rats (300-350 g body weight) received a single intraperitoneal injection of saline (Control group, CTL) or 150 mg/kg alloxan monohydrate (Diabetic group, DM). After 15 days the animals were treated as follows: 1) CTL treated with
saline (C), 2) CTL treated with T3 (T3, 1.5 microg/100 g of body weight/day), 3) DM treated with saline (D), and 4) DM treated with T3 (DT3, 1.5 microg/100 g of body weight/day). After 4 weeks of treatment the animals were sacrificed by decapitation and the hippocampus region subjected to immunobloting technique to evaluate the levels of insulin signaling pathway proteins (IR, Akt and p-Akt, GSK3 and p-GSK3) and neurodegenerative markers (p-Tau and Beta-amyloid peptide). The data were analyzed by ANOVA (two-way) and significance adopted p <0.05. (Protocol CEUA 148/2014). T3 treatment produced a decrease of 33.24% in the IR levels compared to C group. D and T3 groups exhibited a decrease of 38.38% (P<0.05) and 45.27% (P<0.05), respectively, in p-Akt levels compared to C group, while DT3 group showed an increase of 31.32% (P<0.05) and 38.17% (P<0.05) in relation to D and T3 groups, respectively. Increases of 65.26% (P<0.01) in p-GSK3 were observed in T3 group, when compared to C group, while a decrease of 51.51% (P<0.01) was observed in DT3 group in relation to T3 group. Finally, the D group exhibited increases of 94.62% (P<0.01) in p-Tau levels, when compared to C group. In addition, we observed a decrease of 78.92% (P<0.05) in DT3 group when compared to D group. No significant differences were observed in Beta-amyloid levels. Our results suggest that diabetes or increased levels of HT conditions are able to produce an insulin resistance and degenerative states of the rat hippocampus, which may be reversed, at least partially, when both conditions are present.


Poster

483. Alzheimer's Disease: Tau

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 483.21/C7

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Alzheimer's Association NIRG-08-92033

NIH Grant 1R15AG039008

Title: The effect of progranulin haploinsufficiency on an Alzheimer's mouse model

Authors: *C. VOLLERT, L. MARTINEZ, M. TEJADA-SIMON, J. ERIKSEN; Pharmacol. and Pharmaceut. Sci., Univ. of Houston, Houston, TX
Abstract: Alzheimer’s Disease (AD) is the most common cause of neurodegeneration in patients over the age of 65. Pathological hallmarks of AD include the formation of plaques and neurofibrillary tangles in the brain that lead to gross loss of brain function. Mutations in the human GRN gene are associated with frontotemporal lobar dementia with ubiquitinated TDP-43 inclusions. Current studies suggest a reduction in progranulin (PGRN) has a broad importance for neurodegenerative disease and may be a risk factor for AD. Recently, PGRN was shown to be neuroprotective in an AD mouse model and modulates amyloid plaque load. However, despite a growing interest in the role of PGRN in dementia, there are no studies characterizing the behavioral and neuropathological effects of PGRN haploinsufficiency in an AD transgenic mouse model. In this study, we carry out a series of behavioral tests assessing motor control, emotion and memory in an AD mouse model. In addition we examined the effect of PGRN haploinsufficiency on neural correlates of learning and memory and amyloid-associated pathology using immunohistochemistry and western blot techniques. We report that PGRN haploinsufficiency in an AD transgenic mouse model results in significant behavioral impairments.


Poster

483. Alzheimer's Disease: Tau

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 483.22/C8

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Alzheimer's Association ZEN-15-321311 (MNG)

Title: Progranulin over expression in a mouse model of tauopathy

Authors: D. J. FINNERAN1, *M. N. GORDON2, D. MORGAN1, K. R. NASH1;

Abstract: Alzheimer’s disease is a progressive neurodegenerative disorder and the most common form of dementia. Microglial activation and inflammation have been suggested to be significant contributing factors to the neurodegeneration. It has been demonstrated that increasing inflammation exacerbates tau pathology while reducing inflammation ameliorates it. Progranulin is an endogenous growth factor secreted by both neurons and microglia that
attenuates microglial activation. Furthermore, mutations in the GRN gene are associated with Alzheimer’s disease and frontotemporal lobar dementia. Experiments have shown that knockout of GRN exacerbates amyloid-beta deposition and worsens cognitive deficits in a mouse model of APP over expression. Furthermore, haploinsufficiency of GRN in P301L tau transgenic mice increases phosphorylation of tau. Conversely, over expression of progranulin suppresses amyloid-beta deposition in APP mice. The aim of this study was to examine the effects of over expressing progranulin in the Tg4510 mouse model of tauopathy. Four month-old Tg4510 mice were injected bilaterally into the hippocampus and anterior cortex with recombinant adeno-associated virus (rAAV) expressing progranulin or empty capsid as a control virus. Three months later, the animals were assessed behaviorally. We observed no improvement in cognitive performance of the animals injected with rAAV-progranulin as measured by radial arm water maze. Furthermore, the animals injected with rAAV-progranulin ran a significantly greater distance in the open field test than did non-transgenic animals. We are currently examining the levels of neurodegeneration and tau pathology but would predict no improvement or potentially worsening of disease pathology. The lack of behavioral and pathological rescue may be explained by the dual role of progranulin. Progranulin can be cleaved by matrix metalloproteases into one of seven granulins (A through E). Despite progranulin’s anti-inflammatory effects, the granulins act as pro-inflammatory signals. Thus, the over expressed progranulin may have been cleaved to the pro-inflammatory granulins and exacerbated the tau pathology. We are currently examining the levels of progranulin versus granulins in our injected animals. These results indicate that, while progranulin over expression may ameliorate amyloid pathology, over expression does not alleviate tau behavioral deficits in the Tg4510 model of tauopathy.

**Disclosures:** D.J. Finneran: None. M.N. Gordon: None. D. Morgan: None. K.R. Nash: None.

**Poster**

484. Abeta Toxicity

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program/#Poster#: 484.01/C9

**Topic:** C.02. Alzheimer’s Disease and Other Dementias

**Support:** NIH

BrightFocus Foundation

Alzheimer’s Association
Title: Metabotropic glutamate receptor 5 couples brain cellular prion protein physically and genetically to intracellular signaling

Authors: *L. T. HAAS, S. V. SALAZAR, S. M. STRITTMATTER; Yale Univ., New Haven, CT

Abstract: We recently proposed that metabotropic glutamate receptor 5 (mGluR5) propagates neurotoxic signals from extracellular soluble Aβ oligomers (Aβo) bound to cellular prion protein (PrP) onto intracellular substrates. Here we report the novel finding that GPI-anchored PrP interacts physically with intracellular signaling mediators selectively when mGluR5 is present. The physical association between PrP and intracellular proteins can be modified by extracellular soluble Aβ oligomers (Aβo), the key mediators of Alzheimer’s disease (AD) pathophysiology. Genetic coupling between prnp and grm5 mediates acute Aβo-triggered alterations in activation states of intracellular proteins and Aβo-dependent impairment of synaptic plasticity in vitro. Interestingly, we can recapitulate alterations in activation states of intracellular mediators in a chronic in vivo model using APP/PS1 AD transgenic mice. Our data further verifies genetic coupling between prnp and grm5 in mediating changes in protein phosphorylation states selectively in mouse hippocampus and cortex. Importantly, genetic coupling between prnp and grm5 is also responsible for synapse loss and survival deficits in APP/PS1 AD transgenic model mice. Notably, phenotypes in prnp and grm5 double heterozygote transgenic mice are recovered without altered gliosis, β-amyloid plaque load or Aβo levels. This suggests that the recovery is fully dependent on targeting signaling pathways responsible for Aβo-induced aberrations in AD transgenic mice. Thus, this study is of highest significance for the development of AD therapeutics.

Disclosures: L.T. Haas: 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.; Axerion Therapeutics. S.V. Salazar: 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.; Axerion Therapeutics. S.M. Strittmatter: 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.; Axerion Therapeutics.

Poster

484. Abeta Toxicity
**Location:** Hall A  
**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM  
**Program/#/Poster#: 484.02/C10  
**Topic:** C.02. Alzheimer's Disease and Other Dementias  
**Title:** Prnp and Grm5 double heterozygous state rescues Aβo-dependent inhibition of long-term potentiation in hippocampal slices  
**Authors:** *S. V. SALAZAR, L. T. HAAS, S. M. STRITTMATTER;*  
Program in Cell. Neuroscience, Neurodegeneration, and Repair, Yale Univ., New Haven, CT  
**Abstract:** Alzheimer’s disease (AD) is the most common form of dementia, and synaptic dysfunction and loss are central to symptoms of the disease. A growing amount of genetic and biochemical evidence exists for Amyloid-beta (Aβ) as the cause of AD, and it is a soluble aggregated form, oligomeric Aβ (Aβo), as the key toxic species. Our laboratory has identified the cellular prion protein (PrPc) as a high-affinity binding partner to Aβo in an unbiased genome-wide screen. Subsequent work in our lab has identified Aβo-PrPc coupling leads to activation of the intracellular tyrosine kinase Fyn through the transmembrane receptor mGluR5. Further, we have shown that Prnp−/− mice rescue Aβo-dependent deficits in long-term potentiation (LTP). My aim here was to investigate whether haploinsufficiency of Aβo-PrP−/− leads to an amelioration of the inhibitory effect of LTP by Aβo. I used single heterozygous animals, Prnp+/− or Grm5+/−, or transheterozygote Prnp+/− and Grm5+/− animals to determine their effect on LTP in the presence or absence of Aβo. Single heterozygous animals do not rescue Aβo-dependent LTP deficits, while transheterozygote Prnp+/− and Grm5+/− animals rescue these deficits. My work here establishes a genetic interaction between Prnp and Grm5 in the Aβo-dependent inhibition of LTP. These results underscore the importance of mGluR5 and PrPc in developing therapeutics for the treatment of Alzheimer’s disease.  
**Disclosures:**  
**S.V. Salazar:** 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.; Axerion Therapeutics.  
**L.T. Haas:** 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.; Axerion Therapeutics.  
**S.M. Strittmatter:** 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.; Axerion Therapeutics.
484. Abeta Toxicity

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 484.03/C11

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant AG032755

- NIH Grant AG047484
- Alzheimer's Association Art Quilt Initiative (AAQI)
- ADRC Pilot Grant AG005131
- Alzheimer's Association
- NIH Intramural Research Programs of the National Institute on Drug Abuse (NIDA)
- National Institute of Alcohol Abuse and Alcoholism (NIAAA)

Title: Impact of crfr1 ablation on amyloid-β production and accumulation in a mouse model of Alzheimer's disease

Authors: *S. N. CAMPBELL, C. ZHANG, A. D. ROE, N. LEE, K. U. LAO, L. MONTE, M. C. DONOHUE, R. A. RISSMAN; UCSD, La Jolla, CA

Abstract: Stress exposure and the corticotropin-releasing factor (CRF) system have been implicated as mechanistically involved in both Alzheimer's disease (AD) and associated rodent models. In particular, the major stress receptor, CRF receptor type 1 (CRFR1), modulates cellular activity in many AD-relevant brain areas, and has been demonstrated to impact both tau phosphorylation and amyloid-β (Aβ) pathways. The overarching goal of our laboratory is to develop and characterize agents that impact the CRF signaling system as disease-modifying treatments for AD. In the present study, we developed a novel transgenic mouse to determine whether partial or complete ablation of CRFR1 was feasible in an AD transgenic model and whether this type of treatment could impact Aβ pathology. Double transgenic AD mice (PSAPP) were crossed to mice null for CRFR1; resultant CRFR1 heterozygous (PSAPP-R1+/-) and homozygous (PSAPP-R1-/-) female offspring were used at 12 months of age to examine the impact of CRFR1 disruption on the severity of AD Aβ levels and pathology. We found that both PSAPP-R1+/- and PSAPP-R1-/- had significantly reduced Aβ burden in the hippocampus, insular, rhinal, and retrosplenial cortices. Accordingly, we observed dramatic reductions in Aβ peptides and AβPP-CTFs, providing support for a direct relationship between CRFR1 and Aβ.
production pathways. In summary, our results suggest that interference of CRFR1 in an AD model is tolerable and is efficacious in impacting Aβ neuropathology.


Poster

484. Abeta Toxicity

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 484.04/C12

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Pramlintide antagonizes beta amyloid (Aβ)- and human amylin-induced depression of hippocampal long-term potentiation

Authors: *R. KIMURA*¹, D. MACTAVISH², J. YANG²,³, D. WESTAWAY²,³, J. JHAMANDAS⁵;
¹Tokyo Univ. of Science, Yamaguchi, Yamaguchi, Japan; ²Med. (Neurology) and Inst. of Neurosci. & Mental Hlth., ³Ctr. for Prions and Protein Folding Dis., Univ. of Alberta, Edmonton, AB, Canada

Abstract: Alzheimer's disease (AD) is characterized by accumulation of amyloid-β peptide (Aβ) in the brain regions that subserves memory and cognition. We have previously demonstrated that electrophysiological and neurotoxic effects of human amylin and Aβ are expressed via the amylin receptor. Furthermore, the effects of Aβ1-42 and human amylin on hippocampal long-term potentiation (LTP) are blocked by the amylin receptor antagonist, AC253. Recently pramlintide, a synthetic analog of amylin, has been reported to improve cognitive function in transgenic AD mouse models. In this study, we examined the effects of pramlintide on Aβ1-42 and human amylin-evoked depression of LTP at Schaeffer collateral-CA1 hippocampal synapses. In mouse hippocampal brain slices, field excitatory postsynaptic potentials (fEPSPs) were recorded from the stratum radiatum layer of the CA1 area in response to electrical stimulation of Schaeffer collateral afferents. LTP was induced by either high frequency (HFS) or 3-theta burst stimulation (TBS) protocols. Aβ1-42 (50 nM) and human amylin (50 nM), but not Aβ42-1 (50 nM), depressed LTP evoked using both stimulation protocols. Pre-application of pramlintide (250 nM) blocked Aβ- and human amylin-induced reduction of LTP without affecting baseline transmission or LTP on its own. We also examined the effects of pramlintide on LTP in transgenic mice (TgCRND8) that over-express amyloid precursor protein. In contrast to wild-type controls, where robust LTP was observed, 6-12-month old TgCRND8 mice show blunted LTP. In TgCRND8 mice, basal
LTP is enhanced by application of pramlintide. Our data suggest that pramlintide, like AC253, acts as an amylin receptor antagonist to reverse the effects of Aβ_{1-42} and human amylin on LTP and also increases LTP in transgenic mice that demonstrate increased ambient brain amyloid levels. Amylin receptor antagonists may thus serve as potentially useful therapeutic agents in treatment of AD.


**Poster**

**484. Abeta Toxicity**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 484.05/C13

**Topic:** C.02. Alzheimer's Disease and Other Dementias

**Support:** Human Frontiers Science Program

- National Institute for Translational Neuroscience
- Conselho Nacional de Desenvolvimento Científico e Tecnológico
- Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro
- Fundação de Amparo à Pesquisa do Estado de São Paulo
- Canadian Institutes for Health Research
- Canada Research Chair Program

**Title:** Alzheimer-associated Abeta oligomers impact the central nervous system to induce peripheral metabolic deregulation

**Authors:** *N. D. Silva*¹, J. H. R. Clarke², C. P. Figueiredo², R. Frozza¹, J. H. Ledo¹, D. Beckman¹, C. Katashima³, D. Razzoli³, B. Carvalho³, R. Frazão³, M. Silveira³, F. Ribeiro³, T. Bomfim³, F. Neves³, W. Klein⁷, R. Medeiros⁸, F. Laferla⁸, J. Carvalheiro⁸, M. Saad⁸, D. Munoz⁸, L. Velloso⁸, S. Ferreira¹, F. Laferrera;³

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Abstract: Alzheimer’s disease (AD) is associated with peripheral metabolic disorders. Clinical/epidemiological data indicate increased risk of diabetes in AD patients. Here, we show that intracerebroventricular infusion of AD-associated Aβ oligomers (AβOs) in mice triggered peripheral glucose intolerance, a phenomenon further verified in two transgenic mouse models of AD. Systemically injected AβOs failed to induce glucose intolerance, suggesting AβOs target brain regions involved in peripheral metabolic control. Accordingly, we show that AβOs affected hypothalamic neurons in culture, inducing eukaryotic translation initiation factor 2α phosphorylation (eIF2α-P). AβOs further induced eIF2α-P and activated proinflammatory IKKβ/NF-κB signaling in the hypothalamus of mice and macaques. AβOs failed to trigger peripheral glucose intolerance in tumor necrosis factor-α (TNF-α) receptor 1 knockout mice. Pharmacological inhibition of brain inflammation and endoplasmic reticulum stress prevented glucose intolerance in mice, indicating that AβOs act via a central route to affect peripheral glucose homeostasis. While the hypothalamus has been largely ignored in the AD field, our findings indicate that AβOs affect this brain region and reveal novel shared molecular mechanisms between hypothalamic dysfunction in metabolic disorders and AD.


Poster

484. Abeta Toxicity

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 484.06/C14

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: CONACyT Mexico Grant 177269

DGAPA-UNAM Mexico Grant IN200713
**Title:** Pyroglutamate-amyloid-11-42 peptide induces antibodies recognizing main pathological forms of amyloid present in human brain and protection without activation of autoreactive T cells

**Authors:** *G. GEVORKIAN, R. PEREZ GARMENDIA, G. ACERO, A. POMMER, E. GONZALEZ AVILA; UNIVERSIDAD NACIONAL AUTONOMA DE MEXICO, DISTRITO FEDERAL, Mexico*

**Abstract:** Objectives: N-truncated/modified forms of amyloid beta (Abeta) peptide are found in diffused and dense core plaques in Alzheimer’s disease (AD) and Down’s syndrome patients as well as animal models of AD, and represent highly desirable therapeutic targets. An important requirement for safe immunotherapy for AD is prevention of activation of autoreactive T cells since they may increase the incidence of adverse events in the elderly population as observed in early clinical trials. In this study we evaluated antibody production, T cell response and protection in mice and in cholesterol-fed rabbits after immunization with pyroglutamate-amyloid-11-42 (AbetaN11(pE)) in the presence of saponin.

Methods: C57BL/6J wild type and 3XTg-AD mice were immunized with AbetaN11(pE) using saponin as an adjuvant. ELISA and cell proliferation assay followed by flow cytometric analysis and supernatant cytokine measurement were performed using standard protocols. Reduction of Abeta in the brains of AbetaN11(pE)-immunized 3xTg-AD mice as well as cholesterol-fed rabbits was evaluated by ELISA and dot blot.

Results: We have demonstrated that AbetaN11(pE) induced antibodies binding to different pathological amyloid species present in human brain: Abeta1-42, AbetaN3(pE) and AbetaN11(pE). Interestingly, we did not observe proliferation of AbetaN11(pE) immunized mouse T cells in vitro in the presence of any Abeta peptide/epitope probably because of the loss of the known T cell epitope mapped to the central region of Abeta 1-42 in previous studies. We did not detect microhemorrhages in the brains of immunized wild-type and 3xTg-AD mice. Total Abeta load was reduced in the brains of 3xTg-AD mice and cholesterol-fed rabbits after immunization with AbetaN11(pE). Conclusions: Our results may have implication in future design of new immunotherapeutic protocols for AD targeting different Abeta species with reduced risk of side effects related with activation of autoreactive T cells.

**Disclosures:** G. Gevorkian: None. R. Perez Garmendia: None. G. Acero: None. A. Pommer: None. E. Gonzalez Avila: None.
Abstract: Amyloid β peptide (Aβ) is the major component of amyloid fibrils in the senile plaques found in the brains of patients with Alzheimer disease (AD). Aβ aggregation and accumulation cause neuronal cell death, leading to cognitive impairment in patients. The course of Aβ fibril formation starts from a lag phase (nucleation phase), followed by a rapid elongation phase. In this study, we focused on the effects of lipids on Aβ fibrillization. First, we incubated synthetic Aβ1-40 or Aβ1-42 peptides (11 µM) with phospholipid liposomes (PC, PE, PA or PG) or free fatty acids (palmitic acid (16:0), oleic acid (18:1), arachidonic acid (20:4), or docosahexaenoic acid (22:6, DHA)) at 37 °C for 4 h. The level of Aβ fibrillization was quantified using thioflavin T (ThT). Aβ peptides incubated with unsaturated free fatty acids showed higher ThT fluorescence compared to vehicle treated Aβ. Saturated fatty acids and phospholipid liposomes showed no effects. We next incubated Aβ1-40 with vehicle, palmitic acid (saturated) or DHA (unsaturated) for different time periods. DHA treated Aβ showed high ThT fluorescence at 1 h, while vehicle and palmitic acid treated Aβ started the fibrillation at 24 h. This suggests that DHA might shorten the nucleation phase of Aβ fibrillization. Treatment with different concentrations of DHA showed that the effect of DHA was concentration-dependent. Next, we studied whether DHA treated Aβ1-40 had higher seeding effects. We incubated vehicle or DHA treated Aβ1-40 for 24 h, diluted the samples in a new Aβ1-40 mixture at 1/100, and incubated them for a further 4, 8, and 24 h. Surprisingly, DHA treated Aβ1-40 had lower seeding effects compared to vehicle treated samples. To study the morphology of Aβ1-40 fibrils treated with unsaturated fatty acids, Aβ1-40 was incubated with vehicle or DHA for 4, 8, and 24 h, and the ultrastructure was observed by negative stain electron microscopy. DHA treated Aβ1-40 showed short (< 200 nm) and curved fibrils at all time points examined, whereas vehicle treated Aβ formed long (> 200 nm) and straight fibrils at 24 h. In this study, we newly found that unsaturated free fatty acids shorten the nucleation phase of Aβ aggregation, and form Aβ1-40 fibrils with short and curved morphology. These fibrils showed lower seeding effects compared to vehicle treated Aβ1-40. These results suggest that unsaturated fatty acids rapidly convert Aβ peptides into off-pathway intermediates in the Aβ amyloid fibril formation. Further in vitro and in vivo studies are needed to know whether unsaturated fatty acids alleviate or exacerbate the Aβ pathology in the brain.

Disclosures: M. Eto: None. T. Hashimoto: None. T. Shimizu: None. T. Iwatsubo: None.

Poster
Neprilysin: neurogenesis and ß-amyloid toxicity

**Authors:** S. KRAFT, B. HEIMRICH, C. KLEIN, M. MAITRE, H.-D. HOFMANN, A. G. MENSAH-NYAGAN, *M. KIRSCH*

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**Abstract:** Alzheimer Disease is the leading cause of dementia and affects up to 36 Million people. In over 95% of the cases neuronal degeneration is accompanied by pathological accumulation of ß-amyloid resulting from proteolytic processing of APP. There is also evidence, that the decline in cognitive function is exacerbated by impaired neurogenesis. Previous reports relate increased Aß levels to decreased numbers of stem/progenitor cells in the subgranular zone of the dentate gyrus (SGZ) and the subventricular zone of the lateral ventricle (SVZ). The metalloproteinase Neprilysin (NEP) is the key Aß degradating enzyme and by reducing amyloid may also protect neural stem/progenitor cells from its toxic effects. We examined NEP expression in the two major neurogenic areas of the adult mouse brain, SVZ and SGZ, in neural stem cell cultures and in organotypic hippocampal slice cultures (OHSC). Immunofluorescence staining of brain sections shows that NEP is expressed in both neurogenic areas, mainly by immature neurons, identified by staining for doublecortin and PSA-NCAM. In vitro, proliferating neural stem cells express NEP at low levels. However, NEP-expression and activity strongly increased during differentiation. A similar increase of expression during maturation was also observed in OHSCs. By comparing toxicity of exogenous Aß in proliferating and differentiating neural stem cells, we could show that toxicity is inversely correlated with NEP-expression and activity levels. Addition of recombinant NEP completely protects proliferating neural stem cells from Aß-toxicity. In OHSCs exogenous Aß initially induces NEP expression. However, prolonged treatment with Aß, NEP-expression is reduced and plaque like structures develop. In summary, our data suggest that proliferating neural stem cells are particularly vulnerable when exposed to Aß due to their low NEP-expression. Their progeny on the other hand, by upregulating NEP expression become more resistant. Stimulating expression of NEP may therefore help to protect the sensitive stem cell population.

Poster

484. Abeta Toxicity

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 484.09/C17

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: P01NS074969

5K08NS079405

Title: Circadian system influence on beta-amyloid diurnal oscillation

Authors: *G. J. KRESS, F. LIAO, D. M. HOLTZMAN, E. S. MUSIEK;
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Abstract: Alzheimer’s Disease (AD) is the most common neurodegenerative disease associated with age-related cognitive decline. The accumulation of pathogenic β-amyloid (Aβ) plaques during AD progression appears to precede the onset of cognitive impairments. Thus, it is important to identify mechanisms that influence Aβ accumulation which may be useful for the prevention of cognitive decline in AD. Recently, several studies in both humans and rodents, show acute changes in the sleep/wake cycle influence soluble Aβ levels, while chronic sleep/wake disturbances contribute to increased Aβ plaque formation. Additionally, it has been shown that brain Aβ aggregation leads to the disruption of sleep and circadian rhythms in mice. These findings implicate circadian clock dysfunction as either a cause or a consequence of the disease process. Therefore, we sought to investigate the role of circadian clock function in the diurnal oscillation of Aβ levels in a mouse model of AD. Our overall study aim is to disrupt circadian clock function without perturbing the sleep/wake cycle in order to isolate roles of the circadian system impacting brain Aβ levels. We therefore genetically distributed hippocampal circadian clock function via viral mediated deletion of a core clock gene, Bmal1 within the hippocampus of ~1.5 month old AD mice. This AD model has both presenilin 1 (L166P) and the amyloid precursor protein mutations (KM670/671NL), with Aβ plaque deposition at around 2 months of age and rapidly accumulates. Using \textit{in vivo} microdialysis in awake behaving mice, in 12:12 light-dark conditions, we collected hourly interstitial fluid from the ipsilateral virally injected hippocampus. We found no change in the diurnal Aβ oscillation when hippocampal clock function was disrupted, supporting the idea that local clock function within the
hippocampus does not acutely mediate Aβ levels. Next we asked if disrupting circadian clock function within the whole brain, while sparing the suprachiasmatic nucleus (SCN) and leaving the sleep/wake cycle intact, would influence Aβ levels. To our surprise, we found no change in the diurnal oscillation of Aβ levels in the hippocampus. Currently, we are collecting samples to investigate if global Bmal1 gene excision resulting in fragmented sleep patterns impacts the diurnal oscillation of Aβ levels. In summary, our present study shows that acute Aβ levels are not influenced by circadian clock function outside of the SCN, implicating the SCN and sleep/wake dynamics as key regulators of Aβ oscillation. The insight gained from this work should create a cellular framework within which the dynamics of Aβ levels can be better understood and therapeutically modulated.

Disclosures: G.J. Kress: None. F. Liao: 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.; AstraZeneca, Eli Lilly, C2N Diagnostics. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Cure Alzheimer’s Fund, JPB Foundation, Tau Consortium. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Co-founder, C2N Diagnostics LLC and ownership interests. F. Consulting Fees (e.g., advisory boards); AstraZeneca, Genentech, Eli Lilly, Neurophage, C2N Diagnostics. E.S. Musiek: None.

Poster

484. Abeta Toxicity

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 484.10/C18

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH - NIA Grant AG022547

NIH - NIA Grant AG029460

CNPq-Brazil (Post-Doctoral fellowship to AS)

Title: Antibody-assisted determination of molecular mass and shape of neurotoxic Abeta oligomers
Abstract: Alzheimer disease (AD), the most prevalent type of dementia, has been associated with the accumulation of amyloid beta oligomers (AbetaOs) in the brain. Reported AbetaO species vary widely in size, ranging from dimers to larger than 100 kDa. Evidence indicates that not all oligomers are toxic, and there is yet no consensus on the size of the actual toxic oligomer. To address this question, we have made use of conformational antibodies to detect and isolate particular Abeta species from both \textit{in vitro} and \textit{in vivo} sources. In one of these approaches, we have used NU4, a well characterized anti-AbetaO monoclonal antibody, to investigate size and shape of a toxic AbetaO assembly. By using a combination of size-exclusion chromatography and immuno-based detection we isolated an AbetaO-NU4 complex amenable for biochemical and morphological studies. The apparent molecular mass of the NU4-targeted oligomer was 80 kDa. Atomic force microscopy imaging of the AbetaO-NU4 complex showed a size distribution centered at 5.37 nm, an increment of 1.5 nm compared to the size of AbetaOs (3.85 nm). This increment was compatible with the size of NU4 (1.3 nm), suggesting a 1:1 oligomer to NU4 ratio. NU4-reactive oligomers extracted from AD human brain concentrated in a molecular mass range similar to that found for \textit{in vitro}-prepared oligomers, supporting the relevance of the species herein studied. These results, along with data obtained using conformational antibodies targeting distinct Abeta oligomers, represent an important step towards understanding the connection between AbetaO size and toxicity, and may guide the search for novel anti-AD therapeutics aimed to neutralize toxic oligomers.


Poster

484. Abeta Toxicity

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 484.11/C19

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Pfizer-FRQS

Title: The deleterious impact of soluble amyloid-beta oligomers on memory and sleep in Alzheimer's disease
Alzheimer’s disease (AD) is the leading cause of dementia among people over 65 years old, and affects approximately one in three individuals over 85 years. Thirty five million people are affected by AD worldwide, and it is expected to reach 114 million by 2050 if new therapies do not emerge. Currently, there is no treatment to cure or halt this devastating age-related neurodegenerative disorder. Decline in hippocampal-dependent explicit memory (memory for facts and events) is the earliest clinical symptom of AD. It is well established that synapse loss and ensuing neurodegeneration are the best predictors for memory impairments in AD. Latest studies have emphasized the neurotoxic role of soluble amyloid-beta oligomers (Aβo) that begin to accumulate in the human brain approximately 10 to 15 years before the clinical symptoms become apparent. Many reports indicate that soluble Aβo correlate with memory deficits in AD models and humans. In addition, cognitive decline in mild cognitive impairment (MCI) and early AD was shown to be accompanied by poor sleep quality, difficulty initiating sleep, insomnia, and early morning wakening. Although recent ground-breaking discoveries have shown the critical impact of sleep on the regulation of Aβo level in the brain, the interaction existing between soluble Aβo and sleep loss to induce memory impairments at the onset of AD still need to be determined. To achieve our goal, we took advantage of our novel AD animal model in which repeated Aβo injections mimic the synaptic and neuronal loss observed in early AD. We observed that hippocampal accumulation of Aβo was associated with marked cell death, and memory deficits in the passive avoidance task. Electroencephalography (EEG) and electromyography (EMG) measurements were also done on these animals to analyze various oscillatory activities (delta, theta, sigma, alpha, and gamma waves) during rapid eye movement (REM) sleep and non-REM (NREM) sleep as well as in wake AD animals. The results obtained so far support the notion that soluble Aβo might have a deleterious effect on memory and sleep hallmarks affected in AD.

Title: Expression of Calbindin is suppressed by Aβ peptide

Authors: *H. CHOI, E. JUNG, Y. KIM, I. MOOK-JUNG;
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Abstract: Alzheimer’s disease (AD) is an age-related neurological disorder and β-amyloid (Aβ) is one of the major causative factors. Calcium is an important ion to regulate neuronal homeostasis and Calbindin D28k (Calbindin), a calcium-binding buffering protein, has critical roles in the calcium homeostasis and neuroprotection in central nervous system (CNS). It has been reported that Calbindin expression is reduced in the hippocampus of AD mouse models and patients. Moreover, crucial roles of Calbindin in the pathogenesis of AD using Calbindin D28k KO X 5XFAD (CBKOTg) mice were confirmed in our previous study. Since Aβ positive neurons are not able to detect Calbindin expression and Calbindin protein level was reduced by exogenously added Aβ42 in primary cultured hippocampal neurons, we hypothesize that Aβ suppresses Calbindin expression indirectly as Aβ cannot enter the nucleus. In this study, promoter assay system with Calbindin promoter was set up to identify transcription factors that regulate Aβ-induced Calbindin expression. Several possible transcription factors are underway to examine the role of modulation in Calbindin expression. It will provide important clues to figure out the mechanism of Calbindin expression in AD pathogenesis.

Disclosures: H. Choi: None. E. Jung: None. Y. Kim: None. I. Mook-Jung: None.

Poster

484. Abeta Toxicity

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 484.13/C21

Topic: C.02. Alzheimer’s Disease and Other Dementias

Support: NIH/NIA 5R01AG044404

Title: Amyloid-β binds to cerebral proteins

Authors: *D. M. RIDGLEY¹, G. SUN², T. TENG³, J. LEE³;
¹Biochem., ²Bioengineering, ³Univ. of Missouri, Columbia, MO

Abstract: Alzheimer’s disease (AD) is the most common form of dementia which affected an estimated 5.2 million Americans in 2014 and is characterized by neurofibrillary tangles and the accumulation of Amyloid-β (Aβ) forming insoluble aggregates and plaques. Aβ is cleaved from the amyloid precursor protein (APP) by the sequential cleavage of β- and γ-secretases. Both of
the most common forms of Aβ (Aβ42 and Aβ40) are capable of aggregating into oligomers, fibrils and plaques, although Aβ42 is the better aggregator due to two additional aliphatic amino acids on its C-terminus. Increasing evidence demonstrated oligomeric Aβ is the most toxic form of Aβ in AD and this form can lead to oxidative stress and inflammation, two hallmarks of AD. It is this principle that led to the hypothesis that Aβ can bind to or aggregate with natively stable cerebral proteins to form small Aβ-protein complexes that may contribute to AD pathogenesis. Here we introduce the Amyloid-β Binding Affinity Score (AβBAS) which is calculated based on a protein’s secondary structure, hydrophobic amino acid content and disulfide bonds. Over 70 natively stable proteins known to exist within the cerebrospinal fluid were ranked according to AβBAS. Interestingly, some of the highest ranking proteins, such as ApoE and S-100B, are associated with AD pathogenesis. Other high ranking proteins, such as α- and β-hemoglobin, may contribute to AD pathology due to interaction with blood brain barrier (BBB). Finally, new proteins can be hypothesized for their possible involvement in the AD pathology due to their high AβBAS ranking. This study utilizes AβBAS to identify cerebral proteins with a high propensity to bind to Aβ. Furthermore, the affinity for Aβ42 to bind to and/or aggregate with some of the aforementioned proteins is characterized and quantified using spectroscopy and microscopy. Cytotoxic and biochemical assays are also employed to characterize what affect (if any) these Aβ-protein complexes may have on cerebral cellular functions. This study utilizes AβBAS to identify and characterize Aβ42 binding propensity to natively stable proteins providing useful information to understand proteins that are already implicated in AD pathology as well as to unveil new proteins that may interact with Aβ42 and play an unknown role in AD etiology. This work has the potential to introduce new proteins, therapeutic targets and mechanisms that contribute to neurodegeneration in AD.

Disclosures: D.M. Ridgley: None. G. Sun: None. T. Teng: None. J. Lee: None.

Poster

484. Abeta Toxicity

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 484.14/C22

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Role of ABCA7 in clearance of amyloid-beta peptides

Authors: *W. S. KIM;
Neurosci. Res. Australia, Randwick, Australia
Abstract: Genome-wide association studies indicate that ATP-binding cassette transporter A7 (ABCA7) is a strong risk factor for late-onset Alzheimer’s disease (AD). We have previously demonstrated that deletion of ABCA7 in the J20 amyloidogenic mouse causes significant increases in insoluble amyloid-beta levels with concomitant increases in the number of amyloid-beta plaques in the hippocampus. ABCA7 has also been implicated in the role of phagocytosis. However, the mechanism by which ABCA7 reduces amyloid-beta load in the brain is unknown. In this study we investigated the role of ABCA7 in clearance of amyloid-beta (Aβ) peptides. We isolated the brain from ABCA7 knockout mice and wild type littermates following thorough perfusion with Hank’s buffer. The whole brains were homogenized, filtered through cell strainer and cultured in flasks using L929 media. Following shaking the floating microglia were harvested and seeded onto chamber slides. They were then treated with FITC-labelled Aβ40 and Aβ42 oligomers, separately, and the uptake of Aβ oligomers was analysed using fluorescence microscopy. The clearance of both Aβ40 and Aβ42 oligomers was significantly reduced in microglia from ABCA7 knockout mice compared to those from wild type mice. We conclude that ABCA7 mediates microglial clearance of Aβ oligomers, providing a pathogenic mechanism by which ABCA7 is implicated in AD neuropathology.

Disclosures: W.S. Kim: None.

Poster

484. Abeta Toxicity

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 484.15/C23

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: CONACyT 169023

Title: Dependently concentration effects of Amyloid-beta (25-35) peptide on oxidative stress in septal-hippocampal pathway of rats

Authors: *I. LIMON PEREZ DE LEON1, F. SÁNCHEZ-CANO1, A. BÁEZ-CORDERO1, A. PATRICIO1, L. MENDIETA2;
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Abstract: The amyloid-beta (25-35) fraction (Aβ(25-35)) impairs short and long-term memory in rats, similarly to full length amyloid-beta peptide. Moreover, Aβ(25-35) causes a loss of the
cholinergic phenotype of septal neurons without neuronal cell death in medial septum (MS). Therefore the functionality of the septal-hippocampal regions may be crucial for memory process. In this study, we have investigated the effects of three different Aβ25-35 concentrations administered into the medial septum (MS) of rats on spatial memory and nitrosative stress in MS, hippocampus (Hp) and frontal cortex (FCx). For this purpose, male Wistar rats were administered unilaterally with Aβ25-35 at one of the following concentrations [100 µM], [500 µM] and [1 mM] by stereotaxic surgery. Fourteen days after spatial learning was tested in Water maze for 5 consecutive days, and one week later spatial memory was tested during one day of evaluation. At the end of behavioral testing, animals were sacrificed to get the brain and dissect MS, Hp and FCx. In these areas of the brain the following oxidative markers was investigated: Nitric oxide (NO), lipoperoxidation (LPO) by colorimetric methods and nitration of proteins (3-NT) by immunohistochemistry. We found that spatial memory impairments in Aβ25-35- treated group was concentration dependent, where the higher concentration of Aβ25-35 [1mM] causes a significant deficit in the spatial memory respect to control group. Meanwhile [100µM] Aβ25-35 did not impairs the spatial memory of rats. These effect could be due to of that Aβ25-35 [1mM] increases NO and LPO levels, accompanied of higher 3-NT immunoreactivity in MS and Hp respect to animals that received lower doses of Aβ25-35 or control group. These results indicate that Aβ25-35 causes nitrosative stress and impairs spatial memory of rats in a concentration-dependent manner.


Poster
484. Abeta Toxicity

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 484.16/C24

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Alzheimer’s Drug Discovery Foundation 20131002
NIH/NIDA T32 DA007097
Academic Health Center of the University of Minnesota

Title: Aggregation states of amyloid-β affect apolipoprotein E secretion and lipidation
**Authors:** *D. S. CHERNICK*¹, L. LI²;  
¹Pharmacol., ²Exptl. and Clin. Pharmacol., Univ. of Minnesota, Minneapolis, MN

**Abstract:** Alzheimer’s disease (AD) is an age-related debilitating neurodegenerative disease that afflicts approximately five million individuals in the U.S. alone. Although the pathogenesis of AD is not fully understood, it is widely accepted that accumulation of amyloid-β protein (Aβ) in the brain initiates the pathogenic cascade, ultimately leading to neurodegeneration and dementia. Apolipoprotein E (apoE) in the brain is produced primarily by astrocytes and microglia. Once secreted, apoE binds lipids and forms high-density lipoprotein (HDL)-like particles in the interstitial and cerebrospinal fluid. Human apoE has three common isoforms, designated E2, E3, and E4. The apoE4 allele is the major genetic risk factor for late-onset AD, increasing an individual’s risk for AD up to 15-fold. As such, apoE has been extensively studied in AD. It has been shown that apoE directly interacts with Aβ, and that the level and lipidation state of apoE affects Aβ aggregation and clearance. ApoE may also play a role in AD via effects on cholesterol/lipid metabolism, synaptic plasticity, cell signaling, and inflammation. However, the effects of AD pathology on apoE have not been as deeply explored. It has been shown that apoE levels are reduced in AD patients, and that astrocytes surrounding Aβ plaques do not express apoE. Compelling evidence indicates that aggregated states of Aβ play differential roles in AD. However, the effects of different forms of Aβ on apoE have not been thoroughly examined. Therefore, this study was designed to test the hypothesis that different aggregation states of Aβ (monomeric, oligomeric, and fibrilar) will have differential effects on apoE expression, secretion and lipidation in glial cells. Preliminary results show that oligomeric and fibrillar forms of Aβ significantly reduce apoE secretion and lipidation from primary mouse astrocytes and microglia as well as microglial-BV2 cells. Future studies will determine if Aβ has differential effects on the three human apoE isoforms by utilizing primary astrocytes and microglia cultured from targeted replacement mice. Studies of mRNA, apoE-regulating transcription factors, as well as the members of the exocytic pathway will allow us to determine if Aβ’s effect on apoE is through direct effects on expression, or due to indirect effects on the secretory pathway. In addition, both astrocytes and microglia treated with an apoA-I mimetic peptide showed increased secretion and lipidation of apoE. Experiments are underway to determine if treatment with the apoA-I mimetic peptide mitigates Aβ-induced changes in apoE secretion and lipidation.

**Disclosures:**  
*D.S. Chernick:* None.  
*L. Li:* None.

**Poster**  
**484. Abeta Toxicity**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM
Title: Amyloid beta oligomers alter sensitivity of hippocampal neurons to optogenetic stimulation

Authors: *G. J. PAGANDIAZ\(^1\), M. WANG\(^2\), A. JOSE\(^3\), P. SENGUPTA\(^{1,4}\),
\(^1\)Dept. of Bioengineering, \(^2\)Dept. of Animal Sci., \(^3\)Dept. of Mol. and Cell. Biol., \(^4\)Beckman Inst. for Advanced Sci. and Technol., Univ. of Illinois At Urbana-Champaign, Urbana, IL

Abstract: Alzheimer’s disease (AD) is an irreversible, progressive brain disease that slowly destroys thinking skills and memory, and eventually the ability to carry out the simplest everyday tasks. Extra-cellular deposition of insoluble amyloid-β (Aβ) plaques and intra-cellular neurofibrillary tangles in the brains are hallmarks of this disease. The monomers of Aβ peptide are soluble and are thought to be harmless until toxic soluble intermediates, Aβ oligomers (AβOs), concentration is elevated. At relatively lower concentrations, AβOs do not cause apparent neurodegeneration or cell death. But it is postulated that they cause subtle, often undetectable, changes to the neural network dynamics. In this study, we set out to delineate this subtle effects of very low concentrations of AβOs on neuronal network dynamics and its sensitivity to external stimulation. For our investigation, we have developed a hybrid technology based on optogenetics and multi-electrode array (MEA) electrophysiology. This combinatorial approach can be used to induce, detect and track short- and long-term changes in spontaneous and evoked neuronal network activity in response to optical stimuli, using a 470 nm LED, under various types of AβO exposure. We used disassociated cultures of primary mouse hippocampal neurons for detecting any changes in spontaneous network activity. For optogenetic stimulation experiments, mouse hippocampal neurons expressing channelrhodopsin-2 (ChR2) were used. Spike and burst analyses were performed using MC-Rack software (Multichannel Systems), and sensitivity to stimulation was determined using codes developed in-house. Our results demonstrate that even though sub-nanomolar AβOs did not generate any lasting changes to spontaneous network activity, it altered its response to optogenetic stimulation. AβO-treated networks were hyper-sensitive, but they showed larger quiescence time post-stimulation. These observations indicate a subtle, but significant, change in network equilibrium dynamics in cultured networks. A more comprehensive study aiming to link this phenomena to network plasticity, and its potential use as an assay for testing new drug candidates are currently underway.

Disclosures: G.J. Pagandiaz: A. Employment/Salary (full or part-time); University of Illinois at Urbana-Champaign, College of Engineering, Department of Bioengineering. M. Wang: A. Employment/Salary (full or part-time); University of Illinois at Urbana-Champaign, Department of Animal Sciences. A. Jose: None. P. Sengupta: A. Employment/Salary (full or part-time);
Title: Oligomeric Aβ42 toxicity induce ER calcium release in subicular pyramidal neurons

Authors: *S. ANGULO*¹, H. MORENO²;
¹Pharmacol. and Physiol., ²Pharmacol. and Neurol., SUNY Downstate Med. Ctr., Brooklyn, NY

Abstract: Subicular pyramidal neurons are vulnerable to the pathophysiology of Alzheimer’s Disease (AD) in its early stages. There is mounting evidence of calcium dyshomeostasis in AD, but the mechanism of such alterations remains unclear. In familial AD (PS1 mutation), the calcium release from the Endoplasmic Reticulum (ER) is increased through the activation of IP3 receptors (IP3R) and Ryanodine receptor (RyR). It is still unknown if (or how) the oligomeric Aβ42 (oAβ42) affects the function of these receptors in subicular pyramidal neurons. Our preliminary data showed that the acute treatment with oAβ42 increased the peak calcium transients in the apical dendrites of subicular neurons, and we hypothesized that the possible mechanism underlying this process is the increase of calcium release from the ER. We performed calcium imaging in combination with patch-clamp in whole-cell recording in subicular pyramidal neurons from mice (1 month-old) acute brain slices. We used Oregon Green-1 BAPTA (50µM) as a calcium indicator, and studied the calcium transients induced by 3 rapid depolarization steps in voltage-clamp. Calcium signals from the apical dendrite compartment were initially characterized, and pharmacologically dissected. A specific blocker of RyR (Dantrolene 10µM) decreased the peak of the calcium transients by 36%, but the transients remained unchanged in the presence of specific blockers of IP3R (Xestospongin C 1µM, 2-APB 30µM). Treatment with Caffeine (10mM) decreased the peak calcium transient by 46%, possibly through calcium store depletion of the ER. oAβ42 increased the peak calcium transient by 34%, but the combination of oAβ42 and caffeine reduced the peak calcium transient by 40%. Calcium transients were unaltered by the treatment with the control peptide (Aβ scrambled), and were decreased if caffeine was also present in the bath. To verify that caffeine affected the calcium release from the ER in the apical dendrites of subicular pyramidal neurons, we induced calcium transients with rapid and local extracellular application of caffeine (20mM) that were dependent
of the calcium release from ER through RyR. We conclude that the calcium release from the ER (specially the RyR) contribute to the calcium transients evoked by rapid depolarization of subicular neurons. Calcium transients were increased with oAβ42, and this is in part mediated through calcium release from the ER. oAβ42 toxicity in the ER can lead to a molecular cascade of cellular damage that can be part of the early pathophysiology of AD. Presently we are conducting IP3 uncaging experiments in subicular neurons from amyloidogenic mouse model (J20) and control.

Disclosures: S. Angulo: None. H. Moreno: None.

Poster

484. Abeta Toxicity

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 484.19/C27

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: CONACyT scholarship 324341

Title: Aβ1-42 oligomers block the inward rectifier potassium currents from rat pyramidal neurons

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Abstract: A seminal event in the pathogenesis of Alzheimer’s disease (AD) is the abnormal proteolytic processing of amyloid precursor protein (APP), resulting in increased production of a self-aggregating form of β amyloid (Aβ). A portion of Aβ peptides can oligomerize, initially intravesicularly, and be released into the interstitial fluid of brain, where soluble oligomers may diffuse into synaptic clefts and interfere with synaptic function by unknown mechanisms. Aβ oligomers can further polymerize into insoluble amyloid fibrils that aggregate into spherical plaques, resulting in tortuosity and dysfunction of adjacent axons and dendrites in the entorhinal cortex of the brain (Selkoe, 2004). Aβ oligomers are known to be the neurotoxins responsible for neuronal death, the underlying mechanisms remain largely elusive. Here we report the interaction between Aβ1-42 oligomers and inward rectifier potassium channels from pyramidal neurons of the rat entorhinal cortex. Aβ1-42 oligomers were prepared by incubating aqueous solutions of peptide monomer for up to 48 h. Atomic force microscopy showed a wide range of oligomeric species with molecular diameters ranging between ∼15 and 30 nm, corresponding to
roughly 5-40 peptide multimers, which were recognized by 6E10 and OC, sequence- and fibrillar-specific Aβ antibodies, respectively (Kayed et al., 2010). Aβ1-42 oligomers were assayed at 1 µg/ml on the isolated inward rectifier potassium currents (Kir) using both whole-cell and cell-attached voltage-clamp recordings on pyramidal neurons in culture as well as HEK-293T cells that transient expressed Kir2.1 channels. Aβ1-42 oligomers induced a reversible blockade about 40% of the macroscopic current in pyramidal neurons before 5 min application; in HEK cells, we observed a comparable reduction in both open probability and unitary conductance. Finally, we compared the blockade exerted by micromolar concentrations of barium (25 μM) on the Kir currents, and we conclude that Aβ1-42 oligomers and barium have the same molecular target.

**Disclosures:** M. Cuaxospa: None. J.M. Arias: None. U. García: None.

**Poster**

**484. Abeta Toxicity**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 484.20/C28

**Topic:** C.02. Alzheimer's Disease and Other Dementias

**Title:** ROS recovery by HDAC6 inhibition rescues impaired axonal transport by amyloid beta

**Authors:** *H. CHOI, J. KIM, H. KIM, H. CHOI, J. YANG, I. MOOK-JUNG;
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**Abstract:** The major pathological feature of Alzheimer’s disease (AD) is extracellular accumulation of beta-amyloid protein (Aβ). Excessive Aβ have harmful effects to axonal transport in neuronal cells and increase production of reactive oxygen species (ROS), which are responsible for AD. Decreased axonal transport can be recovered through HDAC6 inhibition. In this study, we revealed that anti-oxidant protein peroxiredoxin, one of HDAC6’s substrates, is malfunctioning in AD condition. To further clarify the role of peroxiredoxin in impaired axonal transport by Aβ, we treated Aβ and HDAC6 inhibitor Tubastatin A (TBA) to HT22 and primary neuronal cells. We observed decreased ROS level, followed by reduced Ca2+ level which is increased by Aβ. Moreover, by using live cell imaging method, axonal transport is recovered by TBA and ROS inhibitor. These results indicate that not only α-tubulin, which is a major substrate of HDAC6, but also peroxiredoxin takes part in axonal transport dysfunction. Taken together, we demonstrated malfunctioned peroxiredoxin by Aβ also affects impairment of axonal transport and HDAC6 inhibitor can restore the function of peroxiredoxin which results in rescue of axonal transport.

Poster
484. Abeta Toxicity

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#/Poster#: 484.21/C29

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH/NIA Grant 1R01AG042890

Title: Hsp60 as a protective factor against Amyloid beta misfolding

Authors: *C. MARINO1,2, M. R. MANGIONE3, R. PASSANTINO3, D. BULONE3, P. SAN BIAGIO3, G. TAGLIALATELA1;

Abstract: Alzheimer’s disease (AD) is the most prevalent age-associated neurodegenerative disorder worldwide for which there is no resolving cure available. Although AD is clinically well characterized, the molecular mechanisms responsible for onset and progression of AD remain poorly understood. Understanding such mechanisms is important to reveal new targets for the development of an effective treatment strategy. Among these, the aberrant cleavage of the Amyloid Precursor Protein (APP) by beta and gamma secretase that leads to the formation of the neurotoxic amyloid beta peptide (Aβ) is well documented. In particular, small oligomeric aggregates of Aβ have been shown to be the most toxic contributing, among others, to the induction of mitochondrial dysfunction and neuronal death. Further evidence suggests that age related impairments of such protective mechanisms as chaperones may also contribute to AD progression. Indeed, chaperones like Hsp60, Hsp70 and Hsp90 have been shown to target intracellular amyloid oligomers, thus preventing neuronal damage. Particularly, the mitochondrial Hsp60 seems to play a crucial role in the prevention of Aβ dependent mitochondria dysfunction although the biological mechanisms of Aβ-Hsp60 interaction are poorly understood. Our preliminary data using a cell free model suggest that Hsp60 effectively inhibits Aβ misfolding by directly and irreversibly blocking of its aggregation. On these bases, in the present work we investigated the effect of overexpression of Hsp60 on production and subcellular distribution of Aβ and formation of the toxic Aβ oligomeric species in a more biological relevant system using an established cellular model expressing human APP. For this model,
Chinese hamster ovary (CHO) cells overexpressing human APP751 (7PA2 cells) containing the V717F mutation, that induces the overproduction of Aβ oligomers, were modified to stably overexpress human Hsp60. Western blotting, immunocytochemistry and immunoprecipitation were used to determine Hsp60 and Aβ presence/levels in the whole cell extracts as well as in subcellular fractions and extracellular environments. Our results indicate that overexpression of Hsp60 in 7PA2 cells reduces the intracellular formation of Aβ oligomers without significantly affecting their subcellular localization. Collectively, our data support the hypothesis that Hsp60 reduces levels of the toxic Aβ oligomers and might, therefore, be an effective inhibitor of Aβ neurotoxicity. Further understanding of this biological mechanism could reveal novel targets to develop effective treatment strategies for AD.


Poster

484. Abeta Toxicity

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 484.22/C30

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Recurrent herpes simplex type-1 (HSV-1) infections alter adult hippocampal neurogenesis in mice via amyloid-β protein (Aβ) production and accumulation

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Abstract: Altered neurogenesis in the dentate gyrus (DG) of hippocampus has been suggested to be an early event in Alzheimer’s disease (AD). Though conflicting data have been reported, amyloid-β protein (Aβ) seems to inhibit proliferation and neuronal differentiation of hippocampal neural stem cells (hNSCs). Several studies, including ours, linked recurrent herpes simplex type-1 (HSV-1) infections in the brain to AD based on evidence that HSV-1 triggered APP processing and intracellular Aβ accumulation. Here we checked whether recurrent HSV-1 infections impairs adult hippocampal neurogenesis through Aβ production and accumulation. First, we established a mouse model of recurrent HSV-1 infections in the brain obtained by
inoculating HSV-1 (F strain, 1×10^6 PFU) in 1 month-old C57BL/6 mice via snout abrasion, and periodically reactivating latent virus in the brain by exposing mice to hyperthermia at one month intervals. To study neurogenesis brain sections were immunolabelled for the cell proliferation marker 5-bromo-2’-deoxyuridine (BrdU) and the neuroblasts marker doublecortin (DCX). Infected mice exhibited: i) a significant decrease in the number of DCX⁺, BrdU⁺ and double-labelled DCX⁺/BrdU⁺ cells (-43%, -40%, and -60%, respectively; P<0.05) compared to mock-infected mice; ii) large intraneuronal Aβ accumulation in the DG; iii) memory alterations, documented by a decrease of preference index (from 69±3% to 55±4%; n=5; P<0.05) in the novel object recognition test. To get insights on the mechanisms by which HSV-1 impairs hippocampal neurogenesis, we studied in vitro differentiation of hNSCs isolated from C57BL/6 mice. hNSCs were infected with HSV-1 (0.5 MOI) immediately before starting the differentiation protocol lasting 3, 6 and 9 days (referred as D3, D6 and D9, respectively). The percentages of hNSCs displaying immunoreactivity for the neuronal marker MAP-2 were reduced from 17.5%±1.6% of total cells in mock-infected cultures to 13±1.2% in HSV-1-infected cells at D3, from 24%±2.0% to 12.2±2.3% at D6 and from 34.9%±1.6% to 21.1±2.4% at D9 (P<0.05). Similar reductions were found when hNSCs were exposed to 200 nM Aβ42 during the differentiation protocol, and this effect was independent of apoptotic cell death. The effects of HSV-1 were reversed by adding to the culture medium either 4G8, that is a neutralizing antibody against Aβ, or β- and γ-secretase inhibitors, thus suggesting their dependence on Aβ production and accumulation in hNSCs. Our findings suggest that HSV-1 infection causes intracellular accumulation of Aβ42 in hippocampal NSCs thereby significantly reducing their proliferation and differentiation towards the neuronal phenotype.


Poster

484. Abeta Toxicity

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 484.23/C31

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: KAKENHI26640023

Title: Development of new animal model of Alzheimer's diseases visualizing the intracellular dynamics of the amyloid-β protein
Authors: *T. OCHISHI*, M. DOI, K. YAMASAKI, A. KITAMURA, T. URABE, N. HATTORI, M. KINJO, T. EBIHARA, H. SHIMURA;

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Abstract: The pathology of Alzheimer's disease (AD) is characterized by the extracellular accumulation of amyloid-β (Aβ) peptide and the intraneuronal aggregation of hyperphosphorylated tau protein. In the "β-Amyloid Hypothesis", the extracellular deposit of Aβ peptide plays the central role in the pathogenesis of AD. However, recent studies have suggested that the intraneuronal Aβ peptide is more toxic than extracellular form of it. Because APP is cleaved into several physiologically active protein fragments including Aβ peptide in vivo, they are not rigorous models corresponding to the amyloidosis. Alternatively, direct observation of the process of deposition and disaggregation of Aβ peptide in vivo is quite important for the evaluation of candidate molecules in drug discovery research. With the aim of drug screening by analyzing the intracellular dynamics of Aβ peptide and toxicity in vivo, we have developed the new animal models of AD, an Aβ-GFP mouse and Aβ-GFP C. elegans, which express an Aβ42 peptide fused with GFP in neuronal cells. Observation of Aβ dynamics as GFP fused protein is not easy because the aggregation of Aβ inhibits the fluorescence of fused GFP. However, we succeeded to visualize the Aβ dynamics by arranging the linker sequence between Aβ and GFP. The intracellular Aβ42 was clearly observed as various sized aggregates in the cultured COS7 cells transfected with long linker Aβ-GFP, and brain tissues from Aβ-GFP mouse. Using a fusion protein with the short linker, we could monitor the status of Aβ dynamic in Aβ-GFP C. elegans in vivo. To observe the state of molecule, we compared the aggregate conditions of Aβ-GFP with mutated Aβ-GFP that suppresses the aggregation of Aβ in vivo, using electron microscope and NMR. As results of those, we revealed that the Aβ-GFP is not able to compose fibril but exists in the oligomer state. We compared the intracellular dynamics of these proteins using Fluorescence Correlation Spectroscopy, and revealed that Aβ-GFP composed oligomer in vivo, too. These visualization methods can be applied to analyze the physiological functions of intracellular Aβ, by directly observing the aggregation state and its toxicity in the living animals.


Poster

484. Abeta Toxicity

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM
Program/#Poster#: 484.24/C32

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: ME grant PR2011-0511

- MINECO grant BFU2012-38844
- NIH grant EB000768
- NIH/S10 grant RR025645
- P50 grant AG005134

Title: A topological analysis in APP/PS1 mice reveals that astrocytes do not migrate to amyloid-beta plaques

Authors: *E. GALEA*1,2, W. MORRISON3, E. HUDRY4, M. ARBEL-ORNATH4, B. J. BACSKAI5, T. GÓMEZ-ISLA4, H. E. STANLEY3, B. T. HYMAN4;

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Abstract: Problem: Although the clustering of GFAP immunopositive astrocytes around amyloid-beta plaques in Alzheimer’s disease has led to the widespread assumption that plaques attract astrocytes, recent studies show that astrocytes stay put in injury. Here we re-examine whether astrocytes migrate to plaques by analyzing with mathematical functions, and computer modeling, the topology of astrocytes in 3D images obtained by 2-photon microscopy of living APPSwe/PS1dE9 bitransgenic mice and wild type littermates. Methods: Cranial windows were installed on 5-9-month-old wild type and APPSwe/PS1dE9 mice. Astrocytes were labeled with sulforhodamine, plaques with methoxy-XO4, and vessels with FITC-dextran, and images were obtained by a 2-photon microscope (Olympus Fluoview 1000MPE). Stacks of images were collected 0-200 microns below the pial surface at a 4 micron-step and 1x zoom. Image processing: Astrocytes, plaques and vessels were extracted from 3D reconstructions of such images with algorithms implemented through a combination of the Fiji image processing package and custom Python code. Astrocyte and plaque interactions were then examined with two mathematical functions: the pair-correlation g(r) and the characteristic length (LC) of Voronoi cells. These methods combine global and plaque-centered perspectives, and allow for quantitative comparisons to be made. We used the g(r) function to assess astrocyte topology, and we examined the effect of plaques on several tiers of astrocytes using LC, a very sensitive approach that reveals changes in object position from the redistribution of object-associated domains approximated by Voronoi tessellation. Results: In wild type mice, cortical astrocyte topology fits a model akin to a liquid of hard spheres that exclude each other in a confined space.
Plaques do not disturb this arrangement except at very large plaque loads, but, locally, they cause subtle outward shifts (1-4 microns) of the astrocytes located in three tiers around plaques. Conclusions: The implications of these findings are: (i) that a tight balance of repulsive factors maintains the highly territorial astrocyte organization, and (ii) that, contrary to a belief widely held by Alzheimer’s researchers, astrocytes do not break this order, migrate to plaques, and phagocytose them. We thus conclude that astrocytes respond to plaque-induced neuropil injury mostly by changing phenotype, and hence function, rather than location.


Poster

484. Abeta Toxicity

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program# Poster#: 484.25/C33

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: JO and JR Wicking Trust

Title: Quantifying amyloid-β pathology in an Alzheimer’s disease mouse model: an evaluation of supervised machine learning compared to thresholding

Authors: *M. T. KIRKCALDIE*¹, A. R. O’MARA², A. E. KING², J. C. VICKERS²;
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Abstract: Measuring the load of amyloid plaques in stained or immunolabelled brain tissue images is a technique used in many studies of Alzheimer’s disease. Although thresholding is widely used, it is sensitive to small variations in stain intensity, imaging, and viewing conditions. We studied rater variability in manually thresholding plaque images, selecting 10 subregions from large 8-bit confocal images of 12 month old APP/PS1 mouse cortex, six stained with thioflavin S (thioS) and six immunolabelled with MOAB2 (amyloid β 1-4 epitope, Novus Biologicals). Raters chose thresholds for each subimage three times, in a randomly shuffled order, with no feedback other than the thresholded image. Thresholds chosen for each subimage varied considerably. The average range per subimage, for each rater, was 12.0 levels (SD 9.5) for MOAB2, and 14.9 (SD 10.6) for thioS. Because plaque edges are diffuse, this variation caused large differences in measured plaque load: for subimages with > 0.02% pathology, the ratio of maximum to minimum plaque area for each subimage averaged 1.77 (SD 0.77) for MOAB2, and 1.86 (SD 1.30) for thioS - nearly a twofold difference using thresholds chosen by the same raters.
This variability could be addressed by averaging thresholds, or using an algorithm to choose the threshold. However, no single threshold applied to the large images agreed closely with the raters’ assessment of the subimages taken from it. We applied all possible thresholds (0-255); the best gave an average discrepancy from subimage ratings of 29.6% (SD 27.5) for MOAB2, and 28.3% (SD 28.8) for thioS. A single global threshold, even in the best case, cannot closely reproduce the way human raters judge subregions of the same image. As an alternative, we trained a machine learning algorithm, the random forest classifier (RFC), to identify plaques using the same thresholded subimages as examples, and including image criteria other than pixel intensity. The RFC was then used to segment the large images, and its accuracy was assessed in the same way that global thresholds were evaluated. The RFC segmentations agreed more closely with raters’ thresholded subimages, with discrepancies averaging 13.9% (SD 15.3) for MOAB2, and 22.2% (SD 22.7) for thioS. Similar scores were found when random subsets of raters’ subimages were used for training, implying that RFC segmentation is not as sensitive to which part of the main image is rated in order to train the algorithm. The RFC is available as a free ImageJ plugin, and can be trained using any thresholded or annotated image set. By offering these tools we hope to increase the consistency and comparability of pathology quantification in Alzheimer’s disease research.


Poster

484. Abeta Toxicity

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 484.26/C34

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Allegheny College Neuroscience Program

        Allegheny College Class of 1939 Funds
        Allegheny College James Isherwood Fund

Title: Suppression of hSlo1.1 BK channel current by different Aβ42 conformations

Authors: B. E. ZUECHELKOWSKI¹, *L. B. FRENCH²;
        ¹Neurosci., ²Allegheny Col., Meadville, PA
Abstract: Alzheimer’s disease (AD) is a progressive neurodegenerative disorder characterized by profound memory loss in afflicted individuals. The disease was classically characterized by widespread deposition of insoluble amyloid-beta (Aβ) plaques throughout the cortex, leading to synaptic dysfunction and neuronal loss. Big-conductance potassium (BK) channels are involved in action potential repolarization and calcium-induced apoptosis and have been shown to be affected by Aβ. Recent research has implicated the toxicity of intraneuronal Aβ, particularly soluble oligomers and immature fibrils that have become sequestered in the cell, interfering with synaptic transmission. The current study investigated the effects of different conformations of intracellular and extracellular Aβ on hSlo1.1, a BK channel, in a Xenopus laevis oocyte model. Oocytes expressing hSlo1.1 channels were exposed to intracellular Aβ via dissolution of the peptide in the electrode-filling solution or extracellular Aβ via application to the recording bath solution. BK current was measured by two-electrode voltage clamp. Intracellular fibrils and monomers suppressed BK current without cross-interaction with endogenous oocyte channels, while extracellular fibrils suppressed BK and endogenous current. These results are important for designing an oocyte model in which to study the effects of various Aβ conformations on ion channels.

Disclosures: B.E. Zuchelkowski: None. L.B. French: None.
ameliorate AD. In this study, we have focused on the role of macroautophagy as one cellular clearance pathway that could play a protective role in AD. To model Aβ toxicity in vivo, we have developed an inducible adult-onset Drosophila model of Aβ accumulation with phenotypes including increased mortality and accelerated decline of nervous system function. Here we use genetic over-expression of autophagy-related genes to investigate the effects of neuronal autophagy induction on Aβ accumulation and nervous system decline.

Disclosures: N.S. Woodling: None. J. Castillo-Quan: None. S. Mason: None. L. Partridge: None.

Poster
484. Abeta Toxicity

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 484.28/C36

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Frederick Banting and Charles Best Canada Graduate Scholarship

CIHR

Alzheimer Society of Canada

Title: Amyloid β-induced inhibition of protein prenylation causes autophagy dysfunction

Authors: *K. T. SMITH1,2,3, E. M. GARCIA1,2, A. MOHAMED1,2, E. I. POSSE DE CHAVES1,2,3;

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Abstract: There is ample evidence that autophagy is affected in Alzheimer's disease (AD) but the causes, the nature of the dysfunction and the mechanisms of autophagy impairment are unclear. Autophagy depends on vesicular trafficking and membrane fusion, events that rely on several protein complexes and small GTPases. Previously our lab demonstrated that a neurotoxic mechanism of amyloid-β oligomers (Aβ) is inhibition of protein prenylation. Reduced protein prenylation results in impairment of intracellular and axonal trafficking. The Rab family of small GTPases are prenylated proteins required for normal trafficking, membrane fusion and autophagy. We hypothesize that defective autophagy in AD is due to inhibited protein prenylation and restoring protein prenylation will normalize the autophagic pathway and prevent neuronal death. We are performing in vitro and in vivo experiments to determine the nature of autophagy dysfunction. A biomarker of autophagy is the microtubule-associated protein light
chain 3-II (LC3-II), which associates with the autophagosome membrane. Neurons challenged with Aβ accumulated LC3-II when analyzed by western blot. LC3-II increase is ambiguous, since it could represent induced autophagy or blocked lysosomal degradation of LC3-II. To differentiate between these possibilities we directly examined autophagic flux by expressing mCherry-GFP-LC3, in cultured cells and the mouse CNS. Autophagic flux was decreased in cultured cells treated with Aβ, and was recovered by rescuing protein prenylation with geranylgeranylpyrophosphate. Similarly, simvastatin and psoromic acid, two agents that inhibit protein prenylation, also blocked autophagic flux in a prenylation-dependent manner. Among prenylated proteins we focus on Rab7, which is essential in autophagy progression and lysosomal biogenesis, and is altered in brains of AD patients. During autophagy, Rab7 localizes to autophagosomes together with LC3. Treatment with Aβ reduced Rab7 co-localization with LC3. Normalization of protein prenylation restored colocalization of Rab7 and LC3. Significantly, reversing autophagy dysfunction has been validated as an innovative therapeutic strategy in AD. Yet, the lack of knowledge on the nature and cause(s) of autophagy dysfunction prevents the development of selective autophagy-targeted strategies with disease-modifying value. Our work will provide an essential evidence base for potential therapeutic developments that target autophagy flux in the CNS.


Poster

484. Abeta Toxicity

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 484.29/C37

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: R01NS041202

Title: Degradation of the mdm2/mdm4 cell cycle regulatory protein as a mechanism of amyloid-β-induced neuronal damage

Authors: *C. AKAY, D. J. COLACURCIO, J. W. ZYSKIND, K. L. JORDAN-SCIUTTO; Pathology, Univ. Pennsylvania, Philadelphia, PA

Abstract: Alzheimer Disease (AD) is characterized by the presence of extracellular senile plaques composed predominantly of amyloid-β (Aβ), intracellular neurofibrillary tangles (NFTs) composed of hyperphosphorylated tau protein, and progressive synaptic loss / neuronal death in
the cortex and hippocampus. While the precise mechanisms linking Aβ accumulation to neuronal loss is not completely understood, *in vitro* and *in vivo* studies demonstrate Aβ-mediated activation of the cyclin-dependent kinase 5 (CDK5) plays a role in neuronal death. Intriguingly, aberrant expression of CDK5 as well as several other cell cycle proteins, such as E2F1 and p53 has been reported in several neurodegenerative diseases, including AD. CDK5 activation through activation of the cellular cysteine proteases, calpains is a mechanism of neuronal death. We have recently shown that calpain-mediated degradation of MDMX/MDM4, a cell cycle protein regulating p53 in dividing cells, led to excitotoxic neuronal death. We determined the role of MDMX in *in vivo* and *in vitro* models of Aβ-associated neuronal death. *In vivo* experiments utilized cortical tissue obtained from 14 month-old Tg2576 mice (APPSWE) and age-matched wild-type (w.t.) mice for to assess MDMX protein expression by immunoblotting and immunostaining. *In vitro* experiments utilized 14-21 days *in vitro* primary rat cortical neurons. Aβ-mediated neuronal loss were assessed by MAP2-positive cell counting, and mitochondrial membrane depolarization was determined by ELISA. Small molecule inhibitors to MDMX, and p53, SJ-172550 and pifithrin-α, respectively, were used to dissect the roles of these cell cycle proteins in Aβ-mediated neuronal death *in vitro*. Cortical tissue from 14 month-old APPSWE mice displayed reduced MDMX protein levels relative to w.t. mice, and progressive death of MDMX-expressing cholinergic neurons occurred following the time course of amyloid pathology in these mice. *In vitro*, both Aβ treatment and SJ-172550 led to MDMX loss and neuronal death via calpain and CDK5 activation. Our initial studies also suggest that SJ-172550 induced mitochondrial membrane depolarization independent of p53. These results suggest that MDMX loss may lead to mitochondrial depolarization, calpain and CDK5 activation, contributing to neuronal damage during the progression of AD.


Poster

484. Abeta Toxicity

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 484.30/C38

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: SEP CONACYT CIENCIA BASICA GRANT 220006

Title: Characterization of key regions for the aggregation process that determine the cytotoxic properties of the amyloid beta peptide
Abstract: Alzheimer’s disease is a neurodegenerative disorder and the most common form of senile dementia. Formation of amyloid beta peptide (Aβ) plaques is thought to lead to cell death and loss of cognitive functions. It is known that Aβ aggregation depends on its aminoacid sequence. So by the creation of mutants that can inhibit the conformational change and give rise to stable structures unable to aggregate as an amyloid plaque, it may be possible to identify key residues that affect these processes and use them as therapeutic targets. We decided to study the aggregation process and cytotoxicity of wild type Aβ and different sequence mutants. We studied the aggregation and cytotoxicity of wild type peptides Aβ (25-35) and Aβ (1-40), as well as of the mutants Aβ (1-40) K28A, Aβ (1-40) A30W, Aβ (1-40) M35C, Aβ (25-35) K28A, Aβ (25-35) A30W and Aβ (25-35) M35C using thioflavin T assays, characterization of the aggregates in western blot, and in vitro studies of the effects of the aggregates on cell viability, apoptosis and necrosis of the C6 rat glioblastoma cell line. All mutants showed different aggregation kinetics and cytotoxicity profiles, compared to the wild type peptides. Remarkably, Aβ (1-40) (M35C) mutant showed faster aggregation kinetics, formed more stable aggregates and increased cytotoxicity compared to the Aβ (1-40) wt peptide. We demonstrate the effects of the change on one single aminoacid on cytotoxicity and the aggregation kinetics, showing hot spots in the aggregation process and plaque formation.

Disclosures: V. Zomosa: None. A. Treviño: None. R. Vidaltamayo: None.
Authors: *E. E. Coffey, C. H. Mitchell;* 
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Abstract: A primary cause of familial Alzheimer’s disease (fAD) is mutation of the transmembrane protein presenilin 1 (PS1); recent work has demonstrated a link between the PS1-fAD mutation A246E and lysosomal alkalization. The present study found that cAMP treatment re-acidifies compromised lysosomes in PS1-fAD fibroblasts, and identified a critical role for protein kinase A (PKA) in cAMP-induced re-acidification. When PKA activity was blocked, re-acidification did not occur. Blocking PKA also blocked restoration of cathepsin D active site availability after cAMP treatment, as measured by the reduced binding of boron-dipyrromethene (BODIPY) FL-pepsatin A to the cathepsin D active site. cAMP treatment increased mechanistic target of rapamycin (mTOR) phosphorylation at S2448, a site linked to increased mTOR activity. This mTOR phosphorylation event appears to be an effect, rather than a cause, of lysosomal re-acidification. cAMP treatment reduced mRNA expression of transcription factor EB (TFEB), a critical transcription factor for both lysosomal biogenesis and autophagic regulation; treatment also reduced mRNA expression of one of TFEB’s targets, the v-(H+)ATPase subunit B2. mTOR phosphorylation in response to cAMP was found to depend on PKA activity, as would be expected if mTOR phosphorylation first required successful pH restoration. These data stress the importance of PKA activity to achieve lysosomal re-acidification in PS1-fAD fibroblasts, and suggest that further therapeutic approaches may do well to consider engaging the downstream targets of PKA-dependent re-acidification: mTOR and TFEB. Importantly, cAMP restored lysosomal pH in rat primary cortical cultures following pharmacological pH elevation with tamoxifen; cAMP treatment also blunted the effect of tamoxifen exposure on cathepsin D active site availability in these cultures. Together, these data support a more general role for cAMP in lysosomal re-acidification across multiple cell types, and suggest novel treatment avenues for future work.


Poster

485. Alzheimer's disease: The Secretases

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 485.02/C40

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: FIRB accordi di programma 2011 - RBAP11HSZS

Fondazione Veronesi
Title: ADAM10 endocytosis and Alzheimer’s disease: looking for new therapeutic strategies

Authors: S. MUSARDO¹, S. PELUCCHI¹, D. DI MARINO¹, A. TRAMONTANO³, V. GRIECO², C. GIUDICE², F. GARDONI¹, E. MARCELLO¹, *M. DILUCA⁴,¹;¹Dept. of Pharmacol. and Biomolecular Sci., ²Dept. of Vet. Sci. and Publ. Hlth., Univ. of Milan, Milan, Italy; ³Dept. of Physics, Sapienza Univ. of Rome, Rome, Italy; ⁴Univ. of Milano, Milano, Italy

Abstract: Alzheimer’s disease (AD) is the most common neurodegenerative disorder characterized by progressive loss of synapses and neurons and accumulation of insoluble deposits of amyloid beta-peptide (Aβ). Although AD is emerging as the most prevalent and socially disruptive illness of aging populations, it is currently incurable. Aβ derives from the amyloid precursor protein (APP), which can undergo 2 mutually exclusive pathways in the cell. The amyloidogenic pathway involves BACE and gamma secretase activities and leads to Aβ formation. On the other hand, the main protagonist of the non-amyloidogenic pathway is ADAM10, a disintegrin and metalloproteinase 10, which cleaves APP in the domain corresponding to Aβ, thus precluding Aβ production. Since the modulation of ADAM10 synaptic localization through ADAM10 membrane insertion/removal could constitute an innovative therapeutic strategy to finely tune its shedding activity, we have investigated the ADAM10 endocytosis mechanisms. We show that ADAM10 removal from the plasma membrane is mediated by clathrin-dependent endocytosis and we describe the clathrin adaptor AP2, which initiates the endocytosis process, as new interacting partner of ADAM10 C-terminal domain. In particular, we identify an atypical binding motif for AP2 complex in ADAM10 cytoplasmic tail, which is relevant for ADAM10 endocytosis and the modulation of its plasma membrane levels. Moreover, we describe a pathological alteration of ADAM10/AP2 association in AD patients. On the basis of these findings, we designed four cell permeable peptides (CPPs) able to interfere with ADAM10/AP2 association and, thereby, to reduce ADAM10 endocytosis. We demonstrate, both with in vitro and in vivo experiments, that two of four CPPs are able to disrupt ADAM10/AP2 interaction and to increase the levels of ADAM10 at synaptic membrane. Several studies highlighted the key role of ADAM10 in health and disease, due to its shedding activity toward a number of functional membrane proteins such as APP and N-cadherin. Through its shedding activity, ADAM10 has been shown to regulate key cellular functions including cell growth, adhesion, and migration and spine stabilization in excitatory neuron. A finely balanced membrane level of ADAM10 is an essential prerequisite to control enzyme activity and its functions. We designed a powerful tool able to interfere with mechanisms regulating intracellular trafficking of ADAM10 and to modulate its membrane availability, and thereby to shift APP...
metabolism toward the non amyloidogenic pathway. In light of the above, the use of CPPs is a key starting point to develop new therapeutic strategy for AD.


Poster

485. Alzheimer’s disease: The Secretases

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 485.03/C41

Topic: C.02. Alzheimer’s Disease and Other Dementias

Support: NIH Grant R01NS055223

Alzheimer’s Association Grant IIRG-06-26148

Title: Presenilin-dependent modulation of axodendritic outgrowth requires APP function

Authors: C. DEYTS, M. CLUTTER, S. HERRERA, N. JOVANOVIC, A. GODDI, *A. PARENT;

Univ. Chicago, Chicago, IL

Abstract: Presenilin 1 (PS1) is an essential component of the γ-secretase complex, the enzyme responsible for intramembranous cleavage of amyloid precursor protein (APP) that generates β-amyloid peptides (Aβ) and APP intracellular domain (AICD). Mutations in PS1 lead to dominant inheritance of early onset familial Alzheimer’s disease (FAD). Although there is a consensus that FAD-linked PS1 mutations affect toxic Aβ production, the importance of APP per se and other PS1-dependent substrates in the etiology of the disease has not been confirmed. Recently, we have observed that primary cortical neurons generated from PS1 knock-out (PS1KO) and PS1 knock-in (PS1KI) mice harboring FAD-linked PS1-M146V variant exhibit an increase of axodendritic outgrowth. These outcomes parallel a large and moderate increases of APP-CTF and DCC-CTF in brain lysates prepared from either PS1KO or PS1KI mice, respectively. Accordingly, these results are in support of a partial lost of function of γ-secretase activity in PS1 mutant. Strikingly, lack of APP expression in cortical neurons expressing PS1-M146V variant led to a decrease in both axonal and dendritic outgrowth; an effect that was not seen in neurons lacking DCC expression. These results indicate that APP is required for PS1-dependent change in neurite outgrowth associated with PS1 mutation. Treatment with γ-secretase inhibitor does not
induce additional morphological change in APPKOPS1KI supporting again the importance of APP in PS1-induced neurite outgrowth. Moreover, we observed that accumulation of APP-CTF through concomitant overexpression of APP full-length and γ-secretase inhibition or overexpression of membrane-tethered APP intracellular domain (mAICD) rescue axodendritic outgrowth in PS1KI neurons lacking APP expression. Taken together, our findings provide the first demonstration that a pathological loss of PS1 function lead to a gain of APP function. Our results also identify APP-CTF accumulation as a key player in axodendritic outgrowth. Because accumulation of APP-CTF at the membrane is an invariable outcome of therapeutic inhibition of γ-secretase aimed at reducing cerebral amyloid burden, our findings could have important implications in Alzheimer’s disease treatment. Supported by NINDS and Alzheimer’s Association.

Abstract: Mutations in the genes encoding presenilin 1 (PSEN1), presenilin 2 (PSEN2), and the amyloid precursor protein (APP) are associated with autosomal dominant, early onset familial AD (EOFAD). The PSEN2 N141I mutation causes EOFAD in the Volga German kindred. We have optimized an in vitro protocol to generate human basal forebrain cholinergic neurons (BFCNs) from iPSCs of AD patients and controls. BFCNs are one of the cell types most affected in Alzheimer’s disease patients (AD). We introduced innovations to other published BFCNs protocols including the purification of an intermediate CD271+ forebrain progenitor population by Flow Activated Cell Sorting (FACS) to generate 3D neural embryoid bodies (NEBs). NEBs showed enrichment in BCFNs as compared to unsorted monolayer cultures, demonstrated by higher % of Tuj1+/FOXG1+/ChAT+ neurons. Our immediate goal was to characterize BFCNs and intermediate neural progenitors (NPCs) differentiated from the skin cells of 3 subjects, two affected by PSEN2 N141I mutation, one an unaffected family member control. Skin fibroblasts from these three subjects were reprogrammed, using modified RNA mediated transfer of the reprogramming factors, followed by selection and expansion. All iPSCs were tested to ensure normal karyotypes, robust self-renewal, pluripotency, and expression of stem cell markers including NANOG, TRA-160. After induction of BFCN differentiation in these cell lines, we have analyzed: (1) rates of cell death either at baseline or in response to apoptotic stimuli; (2) capacity to generate Tuj1+/FOXG1+/ChAT+ neurons in vitro; (3) expression of genes/proteins of interest related to neuronal differentiation or inflammation; and (4) generation of soluble and oligomeric Aβ40 and 42. Our ultimate goal is to employ the molecular, transcriptomic, and electrophysiological properties of these cells in the development of novel diagnostics and/or therapeutics for AD. MOV is a New York Stem Cell Foundation-Druckenmiller Fellow. We thank The New York Stem Cell Foundation (MO-V, AAS, SJ, MZ, SAN), NIH AMP U01 AG046170 (MOV, AAS, SJ, MZ, EES, SAN, SG), and Cure Alzheimer’s Fund (MOV, SG, SAN, RET) for their generous support of this research.


Poster

485. Alzheimer’s disease: The Secretases

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 485.05/C43

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: A mutation in presenilin1 promotes Alzheimer-associated phenotype without affecting Notch signaling
Abstract: Neuritic plaques, the unique neuropathological hallmark of Alzheimer’s disease (AD), mainly consist of amyloid-beta protein (Aβ). Aβ is derived from amyloid-beta precursor protein (APP) through sequential cleavages by β- and γ-secretase. Pathogenic mutations in presenilin 1 (PSEN1), the catalytic subunit of the γ-secretase complex, are the major cause of familial AD (FAD). PS1 mutations not only impair APP processing and Aβ generation, but also reduce the cleavage of Notch, one of the most prominent substrates of γ-secretase, leading to Notch signaling disruption. In this study, we found that a novel PSEN1 mutation significantly impairs APP processing and Aβ generation in vitro and in vivo. Moreover, this PSEN1 mutation promotes neuritic plaque formation, and learning and memory deficits in AD transgenic mice. However, the mutant PSEN1 undergoes normal endoproteolysis, and displays normal functions on Notch cleavage and Notch signaling in vitro and in vivo, sparing the impairment of Notch signaling. Taken together, we first demonstrate that a novel mutant PSEN1 functions separately on APP processing and Notch signaling pathway. Furthermore, our study provides a novel insight into developing γ-secretase modulators for AD treatment by specifically modulating APP processing, not affecting Notch cleavage to avoid the severe side effects of γ-secretase inhibitors on Notch signaling.

Disclosures: F. Cai: None. S. Zhang: None. Y. Wu: None. W. Song: None.

Poster

485. Alzheimer’s disease: The Secretases

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 485.06/C44

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant NS047229

NIH Grant AG05138

NIH Grant AG08200

Title: Presenilin1 mutations impair neovascularization and increase vulnerability of brain to ischemia
**Authors:** *A. GEORGAKOPOULOS*¹, Y. YOON², L. CHEN², N. K. ROBAKIS²;  
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**Abstract:** A large amount of evidence has linked brain vascular disorders to the onset of Alzheimer’s Disease (AD). A strong association between cognitive decline and cerebrovascular abnormalities is supported by data that AD brains show impaired brain vasculature (1,2) with changes in the microvasculature preceding neurodegenerative changes and cognitive decline (3,4,5). Insufficient angiogenesis and vascular regression in the AD brain may thus represent an important pathogenic mechanism of disease progression affecting repair of the vasculature and ultimately neuronal health and function. We have observed that Presenilin1 (PS1) a protein involved in the pathogenesis of familial AD (FAD) regulates the angiogenic response of endothelial cells in vitro (6). We want to examine whether PS1 affects vasculature in vivo and whether mutations in PS1 found in FAD impair vascular integrity. To examine the role of PS1 FAD mutations in brain neovascularisation after ischemic insult we induce ischemia using middle cerebral artery occlusion (MCAO) in wild type (WT) and knock in mice carrying PS1 FAD mutations. Lesion size is measured using T2 MRI and restoration of blood volume in the lesion area, which is an indication of neovascularisation, is detected and quantified using perfusion MRI (T1). To identify molecular mechanisms via which PS1 FAD mutations affect brain angiogenesis we perform co-immunoprecipitation experiments in brain extracts of PS1 FAD and WT mice measuring complex formation of angiogenic proteins including VE-cadherin, Rock2 and Raf1 which we have found to depend on PS1 (6). We show that ischemia-induced lesions are larger in brains of PS1 FAD mice compared to WT controls and the edema induced by MCAO remains significantly longer time in the lesion area of the FAD brains compared to WT. GFAP staining shows that accumulation of astrocytes in the lesion area lasts much longer in the PS1 FAD brains indicating a prolonged tissue scar formation compared to WT. Blood volume restoration in the lesion area is also significantly decreased in the brains of PS1 FAD mice compared to WT. Our data show that PS1 mutant mice are more vulnerable to ischemic insult in the brain and that they have reduced ability to recover from this insult compared to WT mice. Interestingly, angiogenic complexes involving VE-cadherin, Rock2 and Raf1 decrease in brains of PS1 knockout (KO) embryos suggesting a role of PS1 in regulation of these complexes in vivo affecting angiogenesis in the brain. Together our data indicate that in FAD, brain vasculature may be compromised due to defective angiogenesis, rendering the brain more vulnerable to toxic insults leading to cell death and neurodegeneration.

**Disclosures:**  A. Georgakopoulos: None. Y. Yoon: None. L. Chen: None. N.K. Robakis: None.

**Poster**

485. Alzheimer’s disease: The Secretases

**Location:** Hall A
Title: Gamma-secretase activating protein (GSAP) alterations in the frontal cortex during the progression of AD

Authors: *M. Nadeem¹, S. Perez², E. J. Mufson³;
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Abstract: Beta-amyloid (Aβ), a pathological hallmark of Alzheimer’s disease (AD), is the product of the concerted cleavage of the amyloid precursor protein (APP) by β- and γ-secretases. However, the molecular mechanism(s) that regulate this process remain unclear. Recent studies suggest that a novel molecule, γ-secretase activating protein (GSAP, 16 kDa), which is derived from a larger 98 kDa precursor protein, regulates γ-secretase activity and Aβ production. However, limited information is available regarding the association between GSAP and the onset of AD. Here we performed quantitative western blotting to determine whether GSAP precursor (GSAPp, 98 kDa) and GSAP (16 kDa) protein levels in frontal cortex, are dysregulated during the progression of AD using tissue obtained from subjects who died with a premortem clinical diagnosis of non-cognitive impairment (NCI), mild cognitive impairment (MCI), mild to moderate AD (mAD) and severe AD (sAD). Our analysis revealed that levels of 98 kDa GSAPp were significantly increased in the frontal cortex in sAD compared to NCI (p=0.004), whereas the levels of 16 kDa GSAP were significantly reduced in sAD compared to MCI (p=0.003) and mAD (p=0.006). Protein levels for 16 kDa GSAP were not associated with mini-mental state examination (MMSE), CERAD, NIA-Reagan and Braak neurofibrillary tangle neuropathology criteria during AD progression. Interestingly, there was a significant negative correlation between GSAPp and MMSE score across the four clinical groups (r=−0.41, p=0.006), but not other clinicopathological variables examined. In summary, our findings indicate that the frontal cortex displays different patterns of GSAPp and GSAP 16 kDa dysregulation late in the disease process. In addition, the association between 98 kDa GSAP levels and MMSE suggest that GSAPp, but not GSAP 16 kDa, plays a role in cognitive decline preferentially late in AD.

Disclosures: M. Nadeem: None. S. Perez: None. E.J. Mufson: None.
**Poster**

485. Alzheimer's disease: The Secretases

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 485.08/C46

**Topic:** C.02. Alzheimer's Disease and Other Dementias

**Support:** NIH R01NS041783

NIH R01NS075346

**Title:** Analysis of Presenilin-1 Asp385 knockin mice

**Authors:** Y. TAN1, D. XIA1, R. KELLEHER2, *J. SHEN3;


**Abstract:** Presenilin-1 (PS1) is the major causative gene of early onset familial Alzheimer's disease (FAD). Presenilin is the catalytic subunit of the γ-secretase complex that cleaves type I transmembrane proteins including APP and Notch. Two conserved aspartate residues (Asp257 and Asp385) in PS1 are thought to constitute the active site of γ-secretase. Overexpression of PS1 with the aspartate to alanine alteration at either site (D257A or D385A) in CHO cells resulted in the failure of mutant PS1 endoproteolysis and abolishment of γ-secretase cleavage of APP. To determine the role of these Asp residues in PS1 in vivo, we generated Psen1 KI mice, in which an Asp385Ala (D385A) alteration was introduced in the genomic Psen1 locus. Interestingly, homozygous D385A KI mice display severe developmental deficits, including perinatal lethality, cerebral hemorrhages, shortened rostro-caudal body axis, and kinked tails; these phenotypes are identical to those of Psen1-null mice. Levels of the Psen1 mRNA are normal in homozygous D385A KI mice, but the N- and C-terminal fragments of PS1 are absent, and PS1 holoprotein accumulates at ~18-fold in homozygous D385A KI mice relative to the wild-type control. Similar to the Psen1-null mutation, the D385A mutation causes severe brain hemorrhages as early as embryonic day 12.5. Biochemical assays of γ-secretase activity in the brain of D385A KI/KI mice revealed abolished γ-secretase-mediated processing of APP, Notch and N-Cadherin. Moreover, histological analysis showed strikingly impaired neurogenesis in D385A KI/KI brains. Collectively, these results demonstrate that the Aspartate 385 of PS1 is indispensable for its function in maintaining normal development and γ-secretase activity.

**Keyword(s):** presenilin, γ-secretase, Alzheimer's disease, knockin mouse

**Supported by grants from the NIH (R01NS041783 to JS, R01NS075346 to RJK)**

**Disclosures: **Y. Tan: None. D. Xia: None. R. Kelleher: None. J. Shen: None.
Attenuation of capacitative calcium entry in familial Alzheimer's disease by gamma-secretase cleavage of stromal interaction molecule 1

Authors: *C. TONG1, C. S. K. LEE2, C. W. H. CHENG2, K.-H. CHEUNG2;
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Abstract: Abnormal γ-secretase cleavage of amyloid precursor protein (APP) to amyloid beta (Aβ) is believed to link with the pathogenesis of Alzheimer's disease (AD). γ-secretase is a multimeric intramembranous protease that cleaves various type-I transmembrane proteins and mounting evidence has demonstrated the proteolytic activity of γ-secretase is presenilin-1 (PS1) dependent. Enhanced γ-secretase activities were observed in many familial AD-linked PS1 mutations and thus leading to disproportionated production of amyloidogenic Aβ42. In addition, these mutations have been demonstrated to disrupt cellular calcium homeostasis by attenuating extracellular calcium (Ca2+) influx through the capacitative Ca2+ entry (CCE) pathway. However, the correlation between CCE attenuation and exaggerated γ-secretase activity is yet to be determined. Using human neuroblastoma SH-SY5Y and single cell imaging techniques, we demonstrated that CCE attenuation was associated with exaggerated γ-secretase activity as application of γ-secretase inhibitor DAPT can restore the attenuated CCE in PS1 mutant expressing cells. Apart from this, we found that DAPT can potentiate CCE in PS1WT expressing and control cell lines, suggesting a negative regulatory role of γ-secretase on CCE. Since stromal interaction molecule 1 (STIM1) is a type-I transmembrane protein and is the ER Ca2+ sensor that participates in the CCE pathway, we hypothesized that STIM1 is a putative substrate for γ-secretase cleavage. Using protein sequence analysis, we found the transmembrane domains of STIM1 and APP were highly conserved, suggesting that STIM1 may be a substrate of γ-secretase. We further validated our hypothesis by in vitro fluorogenic peptide cleavage assay and found that FAD-linked PS1 mutation enhanced not only the γ-secretase cleavage of APP, but also the cleavage of STIM1 that leading to the attenuation of CCE. Furthermore, we demonstrated the neuronal spine maturation in cultured hippocampal neurons were impaired by
FAD-linked PS1 and this impairment can be rescued by γ-secretase inhibitor, DAPT. The result indicated that exaggerated γ-secretase associated with PS1 mutation destabilized mature spine which leading memory loss in AD. In summary, the identification of the γ-secretase cleavage of STIM1 suggests a pathogenic mechanism of FAD, and provides a novel target for therapeutic intervention to treat the disease.


**Poster**

485. Alzheimer's disease: The Secretases

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 485.10/C48

**Topic:** C.02. Alzheimer's Disease and Other Dementias

**Support:** NIH Grant R21AG039596

Alzheimer’s Association 11RG-05-14584

American Health Assistance Foundation A2009045

NIH Grant R181741110

**Title:** Nicastrin and Pen-2 are required while Aph-1 is dispensable for gamma secretase catalyzed turnover of the C-terminal fragment of APP

**Authors:** *C. HU*, T. LI, L. ZENG, M. CUI, X. XU;

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**Abstract:** Based on the “amyloid cascade hypothesis”, the ratio of Aβ42 verses Aβ40 plays a key role in Alzheimer’s disease (AD). The ratio of Aβ42/Aβ40 is controlled by gamma secretase, which cleaves APP (Aβ precursor protein) at C terminal and release Aβ in different length: Aβ38, Aβ40, Aβ42, Aβ43, Aβ46, Aβ49 and so on. Hence, dissecting the biological and biochemical nature of gamma secretase is important in understanding the mechanism of Aβ formation. Gamma secretase is a complex composed of four components: presenilins (PS1 or PS2), nicastrin (NCT), anterior pharynx-defective 1 (Aph-1), and presenilin enhancer 2 (pen-2). The roles of these components remain unclear. Previous studies have suggested that PS functions as the catalytic subunit; NCT may serve as a receptor for the substrate; Aph-1 is assumed to stabilize the other three components; and pen-2 was reported to be essential for the endoproteolysis of PS. However, our recent study revealed that pen-2 is dispensable for the
endoproteolysis of PS, but is required for gamma secretase activity. Our data also demonstrated that NCT is also required for gamma secretase activity. Interestingly, we found that APP is processed by gamma secretase activity in the absence of Aph-1, indicating that Aph-1 is not absolutely required for gamma secretase activity. Furthermore, our data revealed that NCT and pen2 are necessary for a novel post-translational modification of PS1 while Aph-1 is not required. However, the effect of this modification on gamma secretase activity needs to be further investigated.

Disclosures: C. Hu: None. T. Li: None. L. Zeng: None. M. Cui: None. X. Xu: None.

Poster

485. Alzheimer’s disease: The Secretases

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Program#/Poster#: 485.11/C49

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH R01AG025952

NIH R01AG033016

Title: Neuronal overexpression of GGA3 reduces BACE1 levels and BACE1-mediated APP processing in 5XFAD mice

Authors: *W. KIM, G. TESCO;
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Abstract: One of the major pathological hallmarks of Alzheimer’s disease (AD) brains is extracellular amyloid plaques which are mainly composed of the amyloid beta peptide (Aβ). The Aβ is derived by sequential proteolytic cleavage of amyloid precursor protein (APP) via β-site amyloid precursor protein cleaving enzyme1 (BACE1) and γ-secretase. Initial cleavage of APP by BACE1 releases soluble APP (sAPPβ) and generate membrane bound C-terminal fragment (C99). Subsequently, C99 is cleaved by γ-secretase within membrane domain of APP to generate AB. Therefore, BACE1, which is upregulated in AD, is a prime therapeutic target for the treatment of AD patients. It has been shown that BACE1 is normally bound to the trafficking molecules, Golgi-localized gamma-ear-containing ARF binding protein 3 (GGA3) and sorted to the endosomes and lysosomes to be degraded. We had previously shown that levels of BACE1 in AD brains are increased and inversely correlated with GGA3 levels. We had also reported that BACE1 levels are increased in the brain of GGA3 null mice compared with age-matched GGA3
wild type littermates. However, it is unclear whether mice overexpressing GGA3 have any significant effect on BACE1 levels and activity \textit{in vivo}. To address this question, we generated transgenic mice overexpressing N-terminal hemagglutinin tagged human long isoform of GGA3 under transcriptional control of the neuronal-specific mouse Thy1 promoter (GGA3tg+). In this study, we found that BACE1 levels are significantly reduced by the overexpression of GGA3 protein \textit{in vivo} compared with age-matched non-transgenic littermates. Furthermore, in order to examine the effect of reduced BACE1 levels on APP processing in the brain of AD model, we crossed GGA3tg+ mice with mice overexpressing FAD-linked mutation in APP and PS1 under the control of Thy1 promoter (5XFAD). We found that the levels of total APP C-terminal fragments are significantly decreased in the brain of 5XFAD mice overexpressing GGA3 proteins (GGA3tg+:5XFAD). Interestingly, the relative ratio of phosphorylated C99 compared to full-length APP is significantly reduced when compared to the other APP-CTFs in GGA3tg+:5XFAD mice. Our results suggest that the regulation of GGA3 protein could be a potential target to modulate BACE1-mediated APP processing, which is a critical step in A\textbeta formation in AD.

Disclosures: W. Kim: None. G. Tesco: None.

Poster

485. Alzheimer's disease: The Secretases

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 485.12/C50

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: R01AG032432

R42AG031586

R21AG033215

P01AG012411

Title: Soluble APP\alpha decrease tau phosphorylation via BACE1 inhibition and GSK-3\beta-mediated inhibitory phosphorylation

Authors: A. HABIB\textsuperscript{1}, J. DENG\textsuperscript{2}, H. HOU\textsuperscript{2}, D. OBREGON\textsuperscript{2}, S. BARGER\textsuperscript{4}, B. GIUNTA\textsuperscript{2}, Y.-J. WANG\textsuperscript{3}, D. SAWMILLER\textsuperscript{2}, \textsuperscript{*}J. TAN\textsuperscript{2};

\textsuperscript{1}Mol. Pharmacol. and Physiol., \textsuperscript{2}Psychiatry and Behavioral Med., Univ. of South Florida,
Abstract: In Alzheimer’s disease both amyloid and tau pathology accumulates in the brain and causes dementia and cognitive impairment mainly in aged people. Previously we have shown that the non-amyloidogenic APP processing fragment sAPPalpha is neurotrophic and also reduce amyloid pathology from mice brain. In addition, to investigate whether sAPPalpha also reduce tau phosphorylation, we found that inhibition of tau phosphorylation by sAPPalpha is independent of amyloid mediated toxicity. Data from primary cortical neurons and SH-SY5Y cell lines showed that, sAPPalpha increase inhibitory GSK3-beta (Ser9) phosphorylation, which decreased GSK-3-beta activity. In addition, SH-SY5Y cells overexpressing BACE1 (SH-SY5Y/BACE1), as well as HeLa cells overexpressing human tau (HeLa/tau), sAPPalpha increase GSK-3-beta (Ser9) phosphorylation while reducing tau phosphorylation, as indicated by phospho-tau (Thr231) and PHF1 immunoreactivity. These results suggest that sAPPalpha indeed reduces GSK3-beta activity and thereby reduces tau phosphorylation, even in the context of enhanced BACE1 activity. sAPPalpha mediated reduction of tau phosphorylation was not altered by gamma-secretase inhibition. AD mice overexpressing sAPPalpha (PSAPP/TgsAPPalpha) display greater GSK3-beta (Ser9) phosphorylation and less tau phosphorylation than littermate controls (PSAPP). Therefore, sAPPalpha appears to reduce GSK-3-beta activity and thereby reduce tau phosphorylation in vivo as well as in vitro.

Title: BACE expression and activity in rapidly autopsied brains with Alzheimer's disease from Caucasian and Asian patients

Authors: *H. YAO*¹, Y. KONISHI², A. LEVY³, R. LI⁴, Y. SHEN¹; ¹Ctr. for Advanced Therapeut. Strategies for Brain Disorders, Roskamp Inst., Sarasota, FL; ²Dept. of Clin. Research, National Med. Ctr., Tottori, Japan; ³Emory Univ. Med. Sch., Dept. of Neurology, Alzheimer's Dis. Res. Ctr., Atlanta, GA; ⁴Ctr. for Hormones Advanced Sci. and Education, Roskamp Inst., Sarasota, FL; ⁵Beijing Inst. for Brain Disorders, Beijing, China; ⁶Res. Center, Sch. of Life Sciences, Univ. of Sci. and Technol. of China, Hefei, China

Abstract: Elevated β-secretase (β-site amyloid precursor protein-cleaving enzyme1, BACE1) is a major catalytic component of the amyloidogenic cascade. We, as well as two other research groups, discovered that higher levels of BACE activity and protein expression in brains from cases of sporadic AD. Moreover, we recently found that BACE1 activity precedes the clinical diagnosis of AD and could be an early indicator of neuronal dysfunction or pathology in AD. All these results lie in the fact that the population from which our autopsy program was drawn from is overwhelmingly Caucasian. Due to different lifestyle and diet habits between Western and Asian countries, which could be risk factors, in this study, we examined BACE expression and activity in rapidly autopsied brains from Caucasian and Asian patients. We found that not only significantly increased BACE1 activity and protein level occur in Asian AD patients, but also we discovered distinct expression patterns of BACE expression in the AD brains between Caucasian and Asian populations. Moreover, increased BACE1 activity is correlated with plaque numbers and cognition status. Our international and cross-cultural comparative studies of AD offer significant advantages in elucidating risk factors for the disease by providing a wider diversity of environmental exposures as well as greater genetic diversity than do studies confined to a single ethnic group.

Disclosures: H. Yao: None. Y. Konishi: None. A. Levy: None. R. Li: None. Y. Shen: None.

Poster

485. Alzheimer's disease: The Secretases

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 485.14/C52

Topic: C.02. Alzheimer's Disease and Other Dementias
Support: NIH/NIA Grant R01AG032432
NIH/NCCAM Grant R01AT007411
Silver Endowment to J Tan

Title: Swedish mutant APP-based BACE1 binding site peptide reduces APP β-cleavage and cerebral Aβ levels in Alzheimer’s mice

Authors: *D. SAWMILLER1, S. LI1,4, H. HOU1, T. MORI5, A. SMITH6, J. TIAN1, Y. WANG7, B. GIUNTA2, P. R. SANBERG3, S. ZHANG1,8, J. TAN1; 1Developmental Neurobio. Laboratory, Psychiatry, 2Neuroimmunology Lab., 3Neurosurg. and Brain Repair, Univ. of South Florida Med. Sch., Tampa, FL; 4Ctr. for Translational Res. of Neurol. Dis., Dalian Med. Univ., Dalian, China; 5Biomed. Sci. and Pathology, Saitama Med. Univ., Kawagoe, Japan; 6KemPharm, Orlando, FL; 7Neurol., Third Military Med. Univ., Chongqing, China; 8Neurol., Shanghai Hosp., Shanghai, China

Abstract: β-site amyloid precursor protein (APP) cleaving enzyme 1 (BACE1) initiates amyloid-β (Aβ) generation and the resultant cerebral amyloidosis as the characteristic of Alzheimer’s disease (AD). Thus, inhibition of this enzyme has been the focus of a large body of both pre-clinical and clinical research. The most recent clinical trials highlight the difficulty involved in this type of anti-AD therapy as evidenced by side effects that are likely due to the ubiquitous nature of BACE1 which cleave multiple substrates. The human Swedish mutant form of APP (APPswe) has been shown to possess a higher affinity for BACE1 compared to human wild-type APP (APPwt). We pursued a new approach wherein we harness this greater affinity of the substrate to modulate BACE1 APP processing activity. We found that one specific peptide derived from APPswe, containing APPswe β-cleavage sites, strongly inhibits BACE1 activity and thereby reduces Aβ production. This peptide, termed APPswe BACE1 binding site peptide, was further conjugated to the fusion domain of the HIV-1 Tat protein (TAT-APPsweBBP) at the C-terminus to facilitate blood-brain barrier- and neuronal cell-penetration. APPwt and APPswe over-expressing CHO cells treated with this peptide resulted in a marked reduction of Aβ and a significant increase of soluble APPα. Intraperitoneal administration of TAT-APPsweBBP to 5XFAD mice markedly reduced amyloidogenic APP processing and β-amyloid deposits as well as improved hippocampal-dependent learning and memory. These findings provide evidence for a novel strategy for alternative design of BACE1 inhibitors and suggest that TAT-APPsweBBP may be a novel, safe and effective APP substrate-based BACE1 inhibitor for the treatment of AD.

**Poster**

**485. Alzheimer's disease: The Secretases**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 485.15/C53

**Topic:** C.02. Alzheimer's Disease and Other Dementias

**Support:** Roemex Oil Field Chemicals GRANT from private sponsor

**Title:** Brain-specific hBACE1 knock-in induces systemic diabetes via hypothalamic pathology in mice

**Authors:** *K. PLUCINSKA, R. DEKERYTE, D. KOSS, K. SHEARER, N. MODY, G. RIEDEL, M. DELIBEGOVIC, B. PLATT;
Univ. of Aberdeen, Univ. of Aberdeen, Aberdeen, United Kingdom

**Abstract:** Neuronal β-secretase 1 (BACE1) is associated with pathogenesis of Alzheimer’s disease (AD) due to its role in amyloid production exerted via amyloid precursor protein (APP) cleavage. However, growing evidence suggests that BACE1 has multiple substrates other than APP, and its diverse actions may be involved in maintenance of glucose and insulin homeostasis. We here investigated whether introduction of neuronal BACE1 induced changes in central and/or peripheral glucose homeostasis and insulin sensitivity *in vivo*, using our brain-specific human (h) BACE1 knock-in mouse (PLB4). We conducted extensive physiological, biochemical and molecular analyses of systemic and central alterations in glucose metabolism, insulin signalling, lipid and hormonal composition in PLB4 mice using Western blotting, qPCR, immunoassays and LC-MS. Despite a leaner phenotype (~20% body mass loss), PLB4 mice exhibited hyperglycaemic blood (>8mM), severe impairments in glucose clearance from 4 months of age compared to WT counterparts, indicating major metabolic complications and loss of glycaemic control. Hyperinsulinaemia was only detected in 4 and 5-month hBACE1 mice, while low levels of serum insulin in 8-month old transgenics resembled advanced stage of insulin deficiency. The metabolic disturbance in PLB4 mice was associated with advanced hypothalamic endoplasmatic reticulum (ER) stress (elevated expression p-eIF2α and CHOP) and dysregulation of melanocortin system (POMC and MC4R). Brain hBACE1 knock-in promoted defective central insulin signalling and sensitivity (increased expression of PTP1B and insulin resistance-associated RBP4 cytokine). Further, both brain and plasma lipid composition was altered in PLB4 mice, with up-regulation in several classes of lipid species (i.e. phospholipids, triglycerides and ceramides). These hBACE1-induced central alterations paired with initial hyperinsulinaemia promoted impairments in hepatic glycogen storage, fatty liver phenotypes and hepatic insulin resistance. Our data uncover for the first time that neuronal BACE1 regulates...
global metabolic homeostasis and promotes systemic diabetes via induction of hypothalamic ER stress and decreased neuronal insulin sensitivity \textit{in vivo}.


Poster

485. Alzheimer's disease: The Secretases

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 485.16/C54

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: S.A.O./F.R.A. Foundation for Research on Alzheimer's Disease Grant SAO-FRA S#12015

BELSPO IAP Grant P7/16

Title: Transmembrane interactions in APP structure, folding, and processing by \(\gamma\)-secretase

Authors: *P. KIENLEN-CAMPARD\(^1\), C. MARINANGELI\(^1\), M. DECOCK\(^1\), B. TASIAUX\(^1\), J.-N. OCTAVE\(^1\), I. DEWACHTER\(^1\), S. O. SMITH\(^2\), S. N. CONSTANTINESCU\(^1\);

\(^1\)Univ. Catholique De Louvain, Brussels, Belgium; \(^2\)Stony Brook Univ., Stony Brook, NY

Abstract: Objective Understanding the molecular mechanisms controlling APP processing by \(\gamma\)-secretase represents a challenging task in AD research. Transmembrane (TM) interactions appear to have a central role here, both by driving the assembly and activation of the \(\gamma\)-secretase, and the docking/fitting of the substrate prior to cleavage. We identified key structural determinants (GxxxG and GxxxG-like motifs) in APP/Presenilins TM domains (TMDs) and analyzed their role in APP amyloidogenic processing and \(\gamma\)-secretase activity. Methods APP/PS1/PS2 constructs (including FAD mutants) were generated by site-directed mutagenesis. Assembly and activation of \(\gamma\)-secretase was studied by non-denaturating electrophoresis, co-immunoprecipitations, and combined to results of \textit{in vitro} \(\gamma\)-secretase assays. APP transmembrane/juxtamembrane regions were analyzed by structural approaches (FTIR/NMR spectroscopy). APP processing was monitored by electrochemiluminescence assays (ECLIA). Results Gx\(\cdot\)xG motifs in APP TMD were initially found to control the association and orientation of APP homodimers. We showed here they control the \(\alpha\)-helix/\(\beta\)-sheet structure of inhibitory and cholesterol-binding regions, impacting on \(\gamma\)-cleavage and A\(\beta\) release. Mutation of PS1 and PS2 TMD8 motifs either abolishes PS endoproteolysis and \(\gamma\) secretase activity, or...
increase it in the case of FAD mutant. Non-denaturating conditions indicated that GxxxG-like motifs in PS TMD8 are key determinants for the geometry of the mature γ-secretase controlling the switch between physiological and pathological conformations. Conclusion Our data suggest that GxxxG-like motifs in APP and PS are crucial structural determinants for the physiological and pathological processing of APP. They control both the fitness of APP for γ-cleavage and the conformation of the active γ-secretase complex.


Poster

486. Tau and Tauopathies

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 486.01/C55

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: T32 AG000183

R01 AG020670

Belfer Neurodegeneration Consortium

Title: Extracellular mechanisms of tfeb-mediated clearance of ptau in tauopathies

Authors: *H. MARTINI-STOICA¹, H. ZHENG²;
²Mol. and Human Genet., ¹Baylor Col. of Med., Houston, TX

Abstract: Tauopathies affect over thirty million individuals worldwide and the prevalence of Alzheimer’s disease (AD), the most common tauopathy, is expected to quadruple by 2050. Clinically, tauopathies are characterized by progressive cognitive decline and behavioral changes, resulting in total caregiver dependence and death. Pathologically, the disease is marked by intracellular neurofibrillary tangles (NFTs) composed of aggregates of hyper-phosphorylated tau (pTau) protein and extensive neurodegeneration. Unfortunately, current AD therapies addressing tau pathology are limited, lacking in efficacy and/or safety. With studies demonstrating the existence of extracellular tau and cell-to-cell tau spreading, targeting extracellular tau will likely prove crucial to halting disease progression. Our objective is to determine the multiple roles of transcription factor EB (TFEB), a critical regulator of lysosomal biogenesis, in clearing pTau. In addition to neuronal TFEB’s known cell-autonomous effect in
enhancing the autophagy-lysosomal pathway, we hypothesize that astrogial TFEB expression enhances uptake and clearance of aberrant extracellular tau, preventing the neuronal spreading of tau pathology. Upregulation of TFEB in the CNS by adeno-associated virus results in the clearance of aberrant tau and rescue of neurodegeneration in tauopathy mice. Furthermore, TFEB expression in astrocytes plays a functional role in extracellular amyloid-β uptake and clearance. We plan to demonstrate that TFEB upregulation in astrocytes enhances uptake and clearance of pTau \textit{in vitro} and \textit{in vivo}, in addition to halting the propagation of tau pathology \textit{in vivo} tau spreading assay. Through this study, we expect to reveal potential therapeutic targets that will have a substantial impact on AD prevention and treatment.

**Disclosures:** H. Martini-Stoica: None. H. Zheng: None.

**Poster**

**486. Tau and Tauopathies**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 486.02/C56

**Topic:** C.02. Alzheimer's Disease and Other Dementias

**Support:** NIH Grant 1R15AG039008

NIH Grant 1R21NS057651

**Title:** Chronic treadmill exercise prevents tau pathology and behavioral deficits in P301S tau transgenic mice

**Authors:** *J. L. ERIKSEN, O. OHIA-NWOKO;* Pharmacol. and Pharmaceut. Sci., Univ. of Houston, Houston, TX

**Abstract:** Alzheimer’s disease (AD) and some frontotemporal dementias are classified as tauopathies. All tauopathies are characterized by intraneuronal or glial accumulation of fibrillar deposits, which are comprised of hyperphosphorylated and aggregated tau protein. These pathological changes result in significant neurodegeneration in the brain, that results in a progressive decline in cognitive and motor abilities. 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 tau pathology and behavior. Exercise prevented deficits in muscular strength, anxiety-like behavior, sensorimotor gating and cued fear conditioning. Significant reductions in tau...
hyperphosphorylation were observed in the brain, and sarkosyl-insoluble tau was altered in the spinal cord and brain. The effects of exercise on neurodegeneration in the spinal cord and brain were also assessed. We believe this research contributes to a more comprehensive understanding of how treadmill exercise may prevent or mitigate the onset of neurodegenerative disease.

Disclosures: J.L. Eriksen: None. O. Ohia-Nwoko: None.

Poster

486. Tau and Tauopathies

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 486.03/C57

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: CIHR Grant 133693

Alzheimer's Association (USA) NIRG-12-237032

CFI 25026

CIHR Frederick Banting and Charles Best Doctoral Award

Title: Entorhinal tau pathology affects motor behavior but not spatial working memory

Authors: *S. E. TANNINEN*¹, X. JI², A. D. SOKO², R. L. KLEIN³, K. TAKEHARA-NISHIUCHI¹, P. J. FLETCHER²;

¹Psychology, Univ. of Toronto, Toronto, ON, Canada; ²Ctr. for Addiction and Mental Hlth., Toronto, ON, Canada; ³Hlth. Sci. Ctr. Shreveport, Louisiana State Univ., Shreveport, LA

Abstract: Alzheimer’s disease is a progressive neurodegenerative disease characterized by cognitive deficits such as long-term and working memory impairments and non-cognitive abnormalities such as aberrant motor behavior, apathy, and disinhibition. Pathophysiological symptoms of AD are thought to occur years or even decades before the onset of cognitive deficits; however, there is not a consensus for preclinical biomarkers to aid the detection of preclinical AD and the prevention of AD. Post-mortem analyses revealed that the accumulation of hyper-phosphorylated tau in the entorhinal cortex (EC) is one of the first pathophysiological symptoms of preclinical AD. We previously generated a rat model that accurately mimics this site-specific pathology by using a viral vector approach and confirmed that entorhinal tau pathology did not impair the acquisition of a hippocampus-dependent associative memory. The present study aims to identify other potential behavioral deficits that precede the impairment in
long-term memory by examining the behavior of the rat model during an operant delayed non-match to place (DNMTP) task. Adult male Long-Evans rats were trained in DNMTP over 16 weeks. Then, to emulate the site-specific, adult onset pathology observed in human patients with preclinical AD, we transduced with a viral vector, an excess of mutated human tau with the P301L mutation (Tau rats) or green fluorescent protein (GFP rats) into the EC of adult rats. Afterwards, we continued training the rats in DNMTP for 9 weeks during which their working memory and patterns of movement were examined. Tau and GFP rats had similar accuracy in DNMTP at 1, 2, 4, 8, and 16s delays. However, Tau rats, in comparison to GFP rats, showed signs of repetitive or disinhibited motor behaviour: they had more head entries into the food magazine during the inter-trial interval; Tau rats tended to repeatedly nose poke the choice hole after they had chosen the correct choice; and Tau rats moved around more in a novel environment. Thus, tau pathology confined to the EC does not affect spatial working memory but can produce aberrant motor behavior. Non-cognitive symptoms such as repetitive behavior or disinhibition may therefore be a sensitive behavioral measure of preclinical AD.

Disclosures: S.E. Tanninen: None. X. Ji: None. A.D. Soko: None. R.L. Klein: None. K. Takehara-Nishiuchi: None. P.J. Fletcher: None.

Poster

486. Tau and Tauopathies

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 486.04/C58

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NINDS R01-NS076308

Title: Active immunization with highly immunogenic tau epitope induced a strong immune response together with improvement in short memory but failed to significantly reduce tau pathology in a mouse model of tauopathy

Authors: *A. JOLY AMADO¹, H. DAVTYAN², K. SERRANEAU¹, K. ZAGORSKI², M. N. GORDON¹, D. H. CРИBBS³, A. GHOCHIKYAN², N. PETROVSKY², M. G. AGADJANYAN², D. MORGAN¹;

¹USF Hlth. Byrd Alzheimer's Inst., Tampa, FL; ²The Inst. for Mol. Med., Huntington Beach, CA; ³Univ. of California Irvine, Irvine, CA; ⁴Flinders Med. Ctr., Bedford Park, Adelaide, Australia

Abstract: Abnormal tau hyperphosphorylation and its accumulation into neurofibrillary tangles are a hallmark of tauopathies, which are neurodegenerative disorders that include Alzheimer’s
disease (AD). Tau immunotherapy has therefore been proposed as a new therapeutic approach to AD. The aim of this study was to test if active immunotherapy with highly immunogenic tau epitope in a mouse model of tau deposition was capable of reducing levels of tau pathology in the brain and improving cognition. Tg4510 mice, carrying the human four-repeat tau with the P301L mutation (4R0N tauP301L) and the CamK-II tetracycline-controlled transactivator protein were used. Male and female transgenic rTg4510 mice (3 months old; n = 36) subdivided into 3 groups (n=12 per group) received intramuscular injections of tau vaccine, Aβ vaccine or adjuvant only. Non-transgenic and tet only littermates (tetracycline-controlled transactivator protein expressing mice) were used as control groups for behavioral testing and anatomy comparisons. All groups received three injections in alternating weeks and were boosted an additional three times (4 weeks apart) for a total of 7 injections. Mice were subjected to behavioral testing including open field, Y maze, radial arm water maze and novel object recognition by an observer blind to the treatment/genotype of the mice in order to evaluate learning, memory and general activity. Sera were collected at different time points to measure anti-tau and anti-Aβ antibodies responses and tissue was collected at 8 months of age. Active immunization induced strong humoral immune response in both non-transgenic and transgenic mice (1.76 ± 0.17 mg/ml and 1.93 ± 0.27 mg/ml for tau vaccinated mice and Aβ vaccinated mice, respectively). Mice vaccinated with tau epitope displayed an improvement in short-term memory when compared to adjuvant and Aβ treated mice during novel object recognition test. Tau vaccination failed to significantly reduce pathology; however we observed some trend of reduction of total tau and pS396 tau in our study. Altogether, these data indicate that active immunotherapy with tau epitope was effective in improving cognition but had no significant effect on tau pathology in a mouse model of tau deposition. Nonetheless, it is possible that immunotherapy should be initiated very early to be effective in significant reduction of tau pathology. Further analysis and our ongoing studies in different tau transgenic mouse models may result in more comprehensive assessment of vaccine efficacy, and to the understanding of the mechanisms involved in cognition improvement.


Poster

486. Tau and Tauopathies

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 486.05/C59
**Topic:** C.02. Alzheimer's Disease and Other Dementias

**Support:** Internal Institutional Support

**Title:** Examining the intracellular breakdown of toxic tau fragments

**Authors:** A. SHEPARD¹, B. STEVENS¹, E. COOKSEY¹, *M. L. STEINHILB²; ¹Biol., Central Michigan Univ., Mount Pleasant, MI; ²Central Michigan Univ., Mt Pleasant, MI

**Abstract:** Alzheimer’s disease and other tauopathies are characterized by the accumulation of abnormally phosphorylated and aggregated forms of the microtubule-associated protein tau. Several independent laboratories have reported the appearance of a soluble, 17kD fragment of tau in dying neurons that is the product of calpain cleavage. Results from our lab using Drosophila as a genetic model organism for tauopathy show that calpain cleavage of tau has profound impact on neurotoxicity in vivo. Many researchers now support the idea that the toxic tau species is a soluble, highly phosphorylated, aggregated form of tau. What remains unknown is the mechanism controlling how the toxic tau moiety causes neuronal dysfunction. The two major degradation pathways for both physiological and pathological forms of tau are the ubiquitin-proteasome system and the autophagy-lysosome system. Other labs have shown that full-length tau is degraded by the proteasome, but that truncated fragments and soluble oligomers are cleared by autophagy. Accumulating evidence suggests that there is significant cross talk between the autophagic and proteasomal systems and that phosphorylation and truncation may play an important role in targeting proteins to the appropriate degradation pathway. Since others have noted that tau assembly into oligomers inversely correlates with proteasomal degradation (suggesting that soluble oligomers may be degraded via autophagy), we are particularly interested in studying the degradation fate of 17kD tau. We are using primary neuronal cultures to study how wild-type and mutant forms of tau are eliminated by neurons. In this study, Drosophila neurons expressing either human wild-type tau (tauWT), calpain-resistant tau (tauCR), or amino acids 44-230 of human tau comprising the 17kD fragment (tau17kD), were treated with pharmacological agents to manipulate the autophagic and proteasomal systems. Confocal microscopy and live-cell imaging were used to visualize autophagic/proteasomal modulation and assess the toxicity of our tau constructs. Given the importance of soluble tau oligomers and the 17kD fragment, these experiments are important for defining the pathways involved in clearance of the 17kD fragment in order to understand the molecular mechanisms underlying tau toxicity.

**Disclosures:** A. Shepard: None. B. Stevens: None. E. Cooksey: None. M.L. Steinhilb: None.

**Poster**

486. Tau and Tauopathies

**Location:** Hall A
**Title:** Enhanced tau phosphorylation in cold-exposed old mice: linking thermoregulation deficit with Alzheimer's disease

**Authors:** *M. TOURNISSAC*\(^1\,^2\), M. VANDAL\(^1\,^2\), A. FRANCOIS\(^1\,^2\), E. PLANEL\(^1\,^3\), F. CALON\(^1\,^2\);

\(^1\)Axe neurosciences, Ctr. De Recherche Du CHU De Québec, Quebec, QC, Canada; \(^2\)Faculté de pharmacie, \(^3\)Dept. de psychiatrie et neurosciences, Univ. Laval, Quebec, QC, Canada

**Abstract:** Thermoregulatory deficits coincide with a rise in the incidence of Alzheimer's disease (AD) in old age. Lower body temperature increases tau phosphorylation, a neuropathological hallmark of AD. To determine whether old age potentiates cold-induced tau phosphorylation, we compared the effects of cold exposure (4°C, 24 hours) in 18-month-old versus 6-month-old mice. Although reduction of body temperature was similar in young (-4.3%) and old mice (-4.5%), the effect of cold exposure on cortical tau phosphorylation was more pronounced in older mice. Although exposition to 4°C increased tau pSer202 in both 18 and 6-month-old mice (+128%, p<0.03 and +73%, p<0.01, respectively), only old mice displayed a rise in tau pThr181 (+38%, p<0.02) following cold exposure. Furthermore, while tau pSer202 correlated with body temperature in old (\(r^2=0.43\)) and young mice (\(r^2=0.16\)), tau pThr181 and pThr231 correlated with temperature in old (\(r^2=0.47\) and \(r^2=0.28\), respectively) but not in young mice (\(r^2=0.02\) and \(r^2=0.11\), respectively). Interestingly, an increase in pGSK3βSer9 (+49%) was observed only in cold-exposed young mice, suggesting a protective mechanism against cold-induced tau phosphorylation. These results suggest that old age may be associated with a higher susceptibility to a change in environmental temperature, which could contribute to enhance the risk of developing AD.

**Disclosures:** M. Tournissac: None. M. Vandal: None. A. Francois: None. E. Planel: None. F. Calon: None.

**Poster**

486. Tau and Tauopathies

**Location:** Hall A
Title: Neuroprotective effects of a novel Hsp90 C-terminal modulator in Abeta-treated neurons and mutant tau mice

Authors: *M. L. MICHAELIS¹, H. MENCHEN¹, R. PAL¹, H. ZHAO², R. H. SWERDLOW³, E. K. MICHAELIS⁴, B. S. J. BLAGG⁵; ¹Dept Pharmacol, ²Dept Medicinal Chem, Univ. Kansas, Lawrence, KS; ³Dept Neurol., Univ. Kansas, Kansas City, KS; ⁴Dept Pharmacol., Univ. Kansas, Lawrence, KS

Abstract: Most neurodegenerative diseases are characterized by ‘mis-folded proteins’ in aggregates or fibrils, suggesting suboptimal activity of brain molecular chaperones. Heat shock protein 90 (Hsp90) is the master regulator of cell responses to ‘proteotoxic’ stresses, and some Hsp90 modulators can activate transcriptional and/or metabolic cascades leading to protein refolding or degradation. Such modulators have been reported to reduce the amyloid peptide aggregates (Aβ) and hyper-phosphorylated Tau fibrils (P-Tau) induced in animal models of Alzheimer’s (AD). However, most of the Hsp90 modulators were designed to target the N-terminal domain of the protein where they inhibited the ATPase activity and led to in vivo toxicity. We previously described a novel, non-toxic C-terminal Hsp90 modulator, A4, that markedly protected neurons against amyloid peptide (Aβ) - induced toxicity. More recently, we identified a derivative, designated ‘KU-32’, that appears to be even more potent than A4 in protecting neurons against Aβ. The goals of these studies were to test (1) the in vitro and in vivo toxicity of KU-32, (2) the pharmacokinetics, brain permeation, and oral bioavailability of the compound, and (3) the in vivo efficacy of chronic KU-32 treatment in slowing progression of neuritic dystrophy, synaptic abnormalities, levels of P-Tau oligomers, and neuronal cell death in brains of a mouse model with targeted forebrain over-expression of a tet-regulatable P301L Tau mutation, the rTg4510 mice. Our results indicated that KU-32 showed no in vivo toxicity, it readily crossed into the brain, and it was orally bioavailable. Most significantly, chronic administration of KU-32 slowed the accumulation of P-Tau oligomers, reduced the synaptic markers of Tau pathology, and decreased progression of neuronal cell death. In addition, the treatment enhanced retention of a learned task. The data thus far support the potential value of further studies with this C-terminal Hsp90 modulator to assess fully the efficacy of KU-32 in slowing development of the pathology normally resulting from high expression of mis-folded Tau oligomers.
Bone loss as a predictor for brain disease? Decreased bone mineral density and osteoporosis early in the lifespan of htau Alzheimer’s disease mice may be associated with pathology in the dorsal raphe

AD pathological markers have been linked to bone loss and some transgenic strains of amyloid-beta (Aβ) overexpressing mice have an osteoporotic phenotype early in life before the large-scale accumulation of classic transentorhinal AD pathologies are shown. However, it is unclear whether this pathology is due to degenerative changes in central bone regulatory mechanisms or Aβ acting peripherally on the bone itself. We report here, for the first time, early bone loss in an AD model characterized by hyperphosphorylation of tau (ptau). As tau is a cytoskeletal protein primarily expressed in and associated with the nervous system, skeletal deficiencies in these models would be consistent with central pathologies in bone regulatory brain regions. We investigated whether components of this bone regulatory circuitry that include the dorsal raphe nuclei (DRN) and ventral medial hypothalamus (VMH) were affected by ptau pathology early in the htau AD mouse model, and whether this was associated with a skeletal phenotype. Using dual x-ray absorptiometry, we found that at 2-3 months of age, htau mice have significantly lower BMD measured at the lumbar spine, femur, and overall skeleton compared to age-matched wild-type controls. Intriguingly, bone loss was greater in male htau mice as they
met criteria for osteoporosis (BMD more than two standard deviations below control age-matched animal peak bone mass) at the lumbar spine and whole body whereas the female htau mice did not. Additionally, immunohistochemical studies revealed increased staining for ptau-231 and astrogliosis (GFAP) in the DRN of 2-3 month old htau mice compared to wild-type controls. Our results suggest that skeletal deficits in AD may be due to pathologies in central bone regulatory regions of the brain. These findings may be useful for identifying early pathological mechanisms common to bone loss and AD as well as opening new therapeutic windows for delaying or preventing AD.

Disclosures: M.A. Smith: None. D. Margevicius: None. C.M. Dengler-Crish: None.

Poster

486. Tau and Tauopathies

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 486.09/C63

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Behavioral tests identify early phenotypic changes in P301S tauopathy mice

Authors: *L. VER DONCK, M. MAHIEU, K. VAN KOLEN, R. WILLEMS;
Janssen Res. & Development, A Div. of Jans, Beerse, Belgium

Abstract: Tau is a highly soluble cytosolic microtubule binding protein, but under pathological conditions it aggregates into neurofibrillary tangles (NFT) containing hyperphosphorylated tau. Intracellular accumulation of these NFTs is believed to lead to synaptic loss and neuronal cell death, e.g. in Alzheimer’s disease or frontotemporal dementia. Attenuation of tau aggregation therefore holds promise for the treatment of such neurodegenerative diseases. To evaluate the efficacy of potential treatments, animal models are needed where tau aggregation can be correlated to a sensitive behavioral phenotype. The P301S mutant mouse is a commonly used tauopathy model that develops tau aggregation in the CNS along with muscle weakness, tremor and severe paralysis by 3-6 months of age. In this study, we evaluated age-dependent motor dysfunction in female P301S and C57BL6 control (WT) mice using a battery of tests: clasping behavior and paralysis was scored as an indicator of functional loss of hind paws; muscle strength of fore paws was measured as grip strength; the animal’s ability to climb on the top of an inverted grid was used as a measure of muscle strength and motor coordination function; balance was monitored using the beam walking test, measuring the time to reach a platform at the end of either side of a horizontal bar; limb coordination was evaluated on a rotarod by measuring time of first turnaround or when falling off. The mice were evaluated once a month
between 2 and 5 months of age: mice were naïve to the tests at each time point, while one group was re-tested every month. Analysis of data revealed main effects for age, genotype and an interaction effect for age x genotype, except for the grip strength test where only an interaction effect was found. Performance of naïve mutant animals was impaired vs WT in all tests at 4 or 5 months of age, while impairments were already seen in repeatedly tested animals at 3 months in clasping and inverted grid task. Impairment in mutant mice was more pronounced in repeatedly tested mice in grip strength and rotarod. While tau pathology in P301S mice develops at 3 months of age, functional impairments were observed after a short delay suggesting a causative relationship between tau aggregation and the phenotypic changes. More work needs be done to confirm this relationship.


Poster

486. Tau and Tauopathies

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 486.10/C64

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: JSPS KAKENHI Grant Number 26640030

Title: Physiological tissue distributions of tau and MAP2 in mice brains

Authors: *A. KUBO*¹, H. MISONOU², M. MATSUYAMA³, Y. IHARA³, M. TOMOHIRO¹; ¹Dept. of Neuropathology, ²Lab. for Ion Channel Pathophysiology, Doshisha Univ., Kyotanabe-shi / Kyoto-fu, Japan, ³Lab. for Cognition, Memory and Aging, Doshisha Univ., Kyotanabe-Shi / Kyoto-Fu, Japan; ⁴Div. of Mol. Genet., Shigei Med. Res. Inst., Okayama-City, Japan

Abstract: Tau and Microtubule-Associated Protein (MAP)-2, are major MAPs in neurons and are known to stabilize neuronal microtubules (MT). In healthy neurons, it is believed that tau is mainly localized in axons in sharp contrast to MAP2, which localizes in the somatodendritic
compartment. In fact, tau and MAP2 are routinely used as markers of the axon and dendrite, respectively, especially in in vitro studies. However, in contrast to MAP2, as well as to neurofibrillary tangles, the tissue distribution of physiological normal tau in healthy brains is yet inadequately demonstrated because of its poor detectability in immunohistochemistry. To determine the tissue distribution of physiological normal tau, we developed high-sensitive immunohistochemical technique, which allowed us to visualize physiological or pre-pathological tau in the brain tissue. First, we examined the distribution of physiological mouse tau (m-Tau) in the brains of wild-type mice. m-Tau is globally expressed, but abundant in non-myelinated nerve fibers rather than in myelinated nerve fibers in the white matter. The distribution of tau and MAP2 are clearly segregated into axonal and somatodendritic regions, respectively. High-resolution imaging further demonstrated that m-Tau is distributed as punctate along axonal MT. Next, we compared the distributions of endogenous m-Tau and exogenous human tau (h-Tau) in the brains of tau-transgenic (tau-Tg) or tau-knock in (tau-KI) mice. In tau-Tg mice brains, exogenous h-Tau was localized not only in the axonal component but also in the somatodendritic compartments. This was already apparent at a neonatal stage, and further indicates the pre-pathological mislocalization of exogenous tau. In contrast, exogenous h-Tau in tau-KI mice did not show any abnormal sorting. We further found that the MT-binding of h-Tau in tau-Tg mice, but not in tau-KI mice, was affected. Endogenous m-Tau in both of tau-Tg and tau-KI (heterozygous) mice brains behaved as normal throughout their lifetime. Our results indicate the presence of specific mechanisms that somehow regulate the axonal localization and MT-binding of tau, whose expression is driven by the tau promoter but not by ectopic promoter. Tau is hyperphosphorylated and accumulated in cell bodies and dendrites, and forms NFTs in affected neurons of tauopathy brains. In rodent models, similar tauopathy-like pathologies have been reproduced only in the brains of tau-transgenic mice Pathogenesis of exogenous tau in these model mice may be due, at least in part, to the expression of tau using ectopic promoters.


Poster

486. Tau and Tauopathies

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 486.11/C65

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Emergence of early alterations of functional EEG oscillations and network connectivity in a Tau seeding mouse model of Alzheimer’s disease
Abstract: OBJECTIVE: Neuronal network connectivity and oscillations are critical for cognitive functions that are altered in neurodegenerative disorders such as Alzheimer’s disease (AD). Early indicators of AD are crucial for implementing therapeutic interventions when brain systems are still adequately functioning. Here, using a tau seed injection model, the early and late effects of induction and spread of tau aggregation pathology on neurophysiological and functional connectivity networks were investigated.

METHODS: First, sleep-wake and related physiological variables were monitored over 24 weeks in mice equipped with bipolar epidural EEG electrodes. Second, multichannel EEG epidural and intra-hippocampal oscillations were used to assess 1) coherent activity-based synchrony between frontal cortex and CA1-CA3 networks; 2) phase-amplitude cross frequency coupling (CFC) in slow theta and higher gamma EEG frequencies, which are known to be instrumental in cognition, i.e. episodic memory formation; 3) information processing as assessed via auditory evoked potentials (AEP) and evoked oscillations in the passive oddball, mismatch negativity (MMN) paradigm.

RESULTS: Sleep-wake cycle, body temperature and locomotor activity were generally preserved over 24 weeks after the injection of preformed Tau fibrils into the dorsal CA1 region of the hippocampus. In contrast, consistent enhancement of EEG cortical theta oscillations occurred as from week 8 onwards, which coincides with the first signs of neuronal loss, while developing seeded Tau pathology is already at maximal level at 4 weeks. Interestingly, network dynamics analysis revealed weakened functional connectivity between the neocortex and CA1 and drastic impairments in theta-gamma CFC from week 2 onwards, i.e. preceding detectable Tau spread and cell loss. Moreover, the Tau pathology disrupted the P1/N1/P2 AEP complex and evoked oscillations to standard stimuli suggesting a decreased sensory information processing.

DISCUSSION: The present results provide strong preclinical evidence that network alterations indicate very early functional disruptions due to Tau seeding, well in advance of any detectable cognitive or histopathological features. The increase in theta power may disrupt the ability of the brain to dynamically adjust the amplitude of higher frequency oscillations. The strong theta-gamma uncoupling can be exploited as an early cognitive neurophysiological signature of hippocampal network dysfunction. Consequently, network connectivity, theta-gamma CFC and the uncoupling phenomenon described here, might serve as an early, functional biomarker of Tauopathy and AD.

Title: Rho kinase inhibition reduces tau protein level in a Drosophila model of tauopathy

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Abstract: Frontotemporal dementia (FTD) is a neurodegenerative disorder that is as common as Alzheimer’s disease (AD) in the population under age 65 but progresses to death more rapidly than AD. Frontotemporal lobar degeneration that features prominent intracellular aggregates composed of microtubule-associated protein tau is termed FTLD-tau. Aggregated tau can be toxic to neurons, reducing synaptic transmission, promoting synapse loss, and driving cell death. It is proposed that activating intracellular protein degradation pathways may be a rational therapeutic avenue to reduce tau aggregation in FTD. Previous studies indicated that pharmacologic inhibition of Rho-associated coiled-coil containing protein kinases (ROCK) 1 and ROCK2 can enhance protein degradation pathways in mammalian cells. In neurons, we show that treatment with SR3677, a selective drug inhibitor of ROCK2, depletes endogenous tau protein level in a dose-dependent manner. Fasudil, a pan-ROCK inhibitor, had more modest effects. Exposure to SR3677 decreased p62, phospho-mTOR, and phospho-S6 Kinase levels in neurons, suggesting that ROCK2 inhibition promotes autophagy-mediated degradation of tau. Importantly, ROCK2 depletion by shRNA mimicked effects of SR3677 on tau levels as well as autophagy markers in human neuroblastoma cells. ROCK2-shRNA rescue experiments revealed that ROCK2 mutants with amino acid substitutions in the ATP-binding site could not restore tau levels, indicating that ROCK2 kinase activity is required for these effects. Immunoblot analysis of postmortem human frontal cortex tissue samples from FTLD-tau cases, including corticobasal degeneration and progressive supranuclear palsy, revealed changes in autophagy markers as well as ROCK1 and ROCK2 compared to age-matched, pathology-free control cases. Finally, we demonstrate that treatment with Fasudil reduces tau protein level in transgenic expressing human tau. Our findings highlight ROCK2 as a potentially new and exciting therapeutic target to reduce tau protein level in FTD.
**Poster**

486. Tau and Tauopathies

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 486.13/67

**Topic:** C.02. Alzheimer's Disease and Other Dementias

**Title:** The effects of caffeine on *Drosophila* expressing tau pathology

**Authors:** *A. M. BOOTH, A. H. JALALI, D. I. LAMBERCHT, D. D. LENT;* Biol., California State University, Fresno, CA

**Abstract:** Tau pathology commonly occurs in the regions of the brain affected by Alzheimers disease (AD). Current research indicates that caffeine administration in mammalian model organisms such as mice or rabbits that are expressing tau pathology leads to a reduction in both learning and memory deficits and neuronal damage. Additionally, it has been noted that there is a negative correlation of the expression of AD in aging patients with increasing caffeine consumption from beverages such as coffee. The effects of caffeine on tau pathology has not been studied extensively in the more basic model organism *Drosophila.* Studies in *Drosophila* could be useful in developing models to study the basic mechanisms of how caffeine could have an effect on the cognitive deficits and associated neuronal pathology. Genetic tools available in *Drosophila* such as the GAL4-UAS system permits for controlled expression of proteins associated with AD disease. Experiments can be conducted with a large number of flies simultaneously, providing a high throughput model system. Here we examine associative and spatial learning and memory, as well as longevity in *Drosophila melanogaster* expressing human tau protein in the mushroom bodies and ellipsoid body exposed to caffeine post-eclosion in the diet. The mushroom bodies and the ellipsoid body are distinct neuropils in the brain of *Drosophila* that have been implicated in associative and spatial learning and memory and multimodal integration and sensory perception. Additionally, the mushroom bodies and ellipsoid body have been suggested to have homologies to regions of the vertebrate brain attacked during AD (the hippocampus and striatum, respectively). Our approach thus far has focused on analyzing the improvements in the health and lifespan and on the performance in olfactory-shock associative learning and visual place learning assays. Our data suggests that similar to mammalian models of caffeine and AD, caffeine exposure has a positive effect on the behavior and longevity in *Drosophila* expressing tau pathology. By exploring different benefits of caffeine...
on health, spatial cognition and learning, we can better understand the broad deficits caused by tau pathology. Our effort is aimed at further developing new ways to use Drosophila as a high-throughput system to study declines in complex behaviors and the underlying neural deficits associated with AD and other neurodegenerative diseases.


Poster

486. Tau and Tauopathies

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 486.14/C68

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: ANR Cytokalz

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Title: Tau pathology-induced memory impairments: a role for T-cell infiltration?

Authors: *D. BLUM*¹, C. LAURENT¹, G. DOROTHÉ², Y. MONNET³, M. DUCHAMP², A. LEBOUCHER¹, S. BURNOUF¹, R. CAILLIEREZ¹, N. ZOMMER³, D. DEMYER¹, N. JOUY¹, S. SCHRAEN-MASCHKE¹, S. HUNOT³, L. BUÉE¹;
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Abstract: Tau pathology is central to cognitive decline in Alzheimer's Disease. Notably, Tau pathology has been shown to promote synaptic deficits underlying memory impairments in Tau transgenic mice. To which extent immune responses contribute to Tau-induced cognitive decline in AD remains poorly understood. To address this question, we took advantage of the THY-Tau22 mouse model -that progressively develops brain Tau pathology in parallel of cognitive
deficits and reappraised the relationships between central immune response and Tau lesions. Using conventional immunohistochemical approaches as well as transcriptomics, we observed, as expected, astro/microglial hippocampal activations spatially and temporally correlated with Tau pathology. More surprisingly, we also observed that several markers of adaptive immune responses were upregulated in Tau transgenic mice. We identified, for the first time, CD8+ T-cell infiltration in the brain of Tau mice, without any major impairment of the blood brain barrier integrity. T-cell infiltration was associated with upregulation of chemokine production (Ccl3, Ccl4, Ccl5) as observed by a combination of transcriptional, biochemical and immunohistochemical approaches. In order to get insights into the potential implication of T-cell infiltration in the pathophysiological development in THY-Tau22 transgenic mice, we chronically depleted T-cells using anti-CD3 antibodies. Such treatment totally prevented CD8+ T-cell infiltration in the brain of Tau mice and restored normal spatial memory as measured using the Y-maze task. Overall, our study highlights the involvement of chemokines and adaptive cellular immunity in the development of memory deficits in Tau pathologies.


Poster

486. Tau and Tauopathies

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 486.15/C69

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: the Strategic Research Program for Brain Sciences of the Ministry of Education, Culture, Sports, Science and Technology of Japan

CREST/JST

Title: The loss of FUS leads to brain atrophy accompanied with neuronal loss

Authors: *Y. FUJIOKA¹, S. ISHIGAKI¹, S. YOKOI¹, D. HONDA¹, T. UDAGAWA¹, H. OKADO², M. YOSHIKAWA¹, A. TAKASHIMA¹, H. WATANABE¹, M. KATSUNO¹, G. SOBUE¹;
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Abstract: [Background] FUS is a causative gene for familial ALS and FTLD. FUS aggregates are recognized as a pathological hallmark of both familial and sporadic ALS/FTLD. In ALS/FTLD, distinct atrophies are the major characteristics in the lesion with FUS pathology. [Aim] To determine whether FUS knock-down causes neuronal loss and brain atrophy by using a FUS knock-down mouse model established by injecting adeno-associated virus (AAV) encoding shRNA against FUS into the bilateral hippocampus, which exhibited FTLD-like behavioral impairments. [Methods] We injected AAV encoding shRNA against FUS (shFUS) and control (shCont) to the bilateral hippocampus at the age of 6 weeks. The size of hippocampus was measured at 6-month, 12-month and 18-month of post-injection by 3.0T MRI. Immunohistochemical analysis was also performed to validate the results of imaging study. [Results] We observed a significant decrease in hippocampal volume in shFUS mice at 18 months of post-injection compared to shCont, whereas no apparent difference was observed until 12 months of post-injection. Marked hippocampal atrophy accompanied with neuronal loss and subsequent enlargement of the lateral ventricles were observed in shFUS mice at 18 months of post-injection, whereas histology was almost intact at 12 months of post-injection. [Conclusion] The loss of FUS leads to brain atrophy accompanied with neuronal loss in an age-dependent manner. Taken together with their behavioral impairments, our FUS knock-down animals mimic the phenotypes of FTLD.


486. Tau and Tauopathies

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 486.16/C70

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NHMRC

Title: Targeting the mapt locus by TALEN to study the trafficking of endogenous tau

Authors: *D. XIA, J. GOTZ;
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**Abstract**: Neurofibrillary tangles (NFTs) are a major histopathological hallmark of neurodegenerative disorders such as Alzheimer’s disease (AD) and frontotemporal dementia. Their major proteinaceous constituent is tau, a microtubule-associated protein tau (MAPT). Tau is enriched in neurons, where under normal conditions, it is mainly localized to the axon, while expression in the dendrites is weak. In contrast, when Tau is overexpressed in cultured neurons it distributes uniformly in all processes. It has been hypothesized that in AD, Tau relocalizes from the axonal to the somato-dendritic domain and that this process is facilitated by another AD hallmark, amyloid-β. Studying tau however is compromised by classical over-expression approaches, because the Tau-encoding mRNA is translated in the soma whereas in the case of endogenous Tau, its mRNA is transported to the axonal shaft for local translation. Also, tau exists in several isoforms. To overcome the drawback of overexpression system, we established a transgenic mouse line targeting a photo-convertible mEOS2 tag to the carboxy-terminus of the MAPT gene, using the genome-editing tool TALEN. Live cell imaging techniques will allow monitoring the trafficking of endogenous Tau in neurons under both physiological and pathological conditions.

**Disclosures**: D. Xia: None. J. Gotz: None.

**Poster**

486. Tau and Tauopathies

**Location**: Hall A

**Time**: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#**: 486.17/C71

**Topic**: C.02. Alzheimer's Disease and Other Dementias

**Title**: Extracellular levels of tau protein, β-amyloid and neurotransmitters in cerebral structures of a mouse model of Alzheimer’s disease

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**Abstract**: Background Alzheimer’s Disease (AD) pathology is characterized by β-amyloid (Aβ) deposits and neurofibrillary tangles (NFT). The pathology progresses from the entorhinal cortex to hippocampal sub-regions and the cortical area via anatomical connections. It is
hypothesized that progression occurs via a cell to cell transmission and tau is secreted into the extracellular space. Therefore, progression of the pathology can be evaluated using a microdialysis approach. The present study monitors the different forms of β-amyloid, tau protein, monoamines, acetylcholine (ACh) and kynurenate (KYN) levels in the interstitial fluid (ISF) of three different brain structures of a transgenic mice strain exhibiting Aβ and tau pathology.

**Methods** Male TauPS2APP (APP695[K670N-M671L]/PS2N141I/TauP301L (3xtg)) were evaluated at 8 months in comparison to C57Bl6 mice. A ~300kDa cut off membrane, (Brainlink, Netherland) was stereotaxically implanted in the frontal cortex (2 mm long, Lat, -0.4, AP, +2.0, DV, -3.0), the ventral hippocampus (2mm long, Lat, +2.9, AP, -3.1, DV, -4.5) or the amygdala region (1 mm long, Lat, +2.5, AP, -1.5, DV, -5.5) under ketamine/xylasine anaesthesia. Two days later, freely-moving mice were perfused (0.5µl/min) with Ringer solution and samples collected during 7 hours. Simultaneous ELISA quantification of Aβ1-40, Aβ1-42 and total tau was performed using MesoScale Discovery technology. Neurotransmitters were evaluated by HPLC coupled to electrochemical, fluorimetric detection or mass spectroscopy. **Results** ISF levels of total tau were significantly increased in the ventral hippocampus of the 3xtg mice compared to controls. The similar increase was observed in the frontal cortex and the amygdala region. ISF levels of Aβ1-40 and Aβ1-42 were similarly increased in the 3xtg mice. In contrast, levels of noradrenaline were significantly reduced in the ventral hippocampus, frontal cortex and the amygdala region of the 3xtg mice. No significant differences were observed in serotonin, ACh and KYN levels. **Conclusions** Monitoring tau, β-amyloid, monoamine, KYN and ACh levels in different brain regions using microdialysis provides a useful tool to assess the spreading of the AD pathology and neuronal dysfunction. The present research was part of the PharmaCog consortium funded by the European Community's Seventh Framework Programme for the Innovative Medicine Initiative under Grant Agreement n°115009. All procedures using these animals conformed to international European ethical standards (86/609-EEC) and the French National Committee (décret 87/848) for the care and use of laboratory animals.

**Disclosures:** E. Schenker: A. Employment/Salary (full or part-time); Institut de Recherches Servier. G. Rollin-Jego: A. Employment/Salary (full or part-time); Institut de Recherches Servier. R. Billiras: A. Employment/Salary (full or part-time); Institut de Recherches Servier. V. Pasteau: A. Employment/Salary (full or part-time); Institut de Recherches Servier. J.C. Richardson: A. Employment/Salary (full or part-time); GlaxoSmithKline R&D. S. Dix: A. Employment/Salary (full or part-time); Eli Lilly. C. Czech: A. Employment/Salary (full or part-time); Roche Pharma and Early Development. L. Ozmen: A. Employment/Salary (full or part-time); Roche Pharma Research and Early Development. A. Gobert: A. Employment/Salary (full or part-time); Institut de recherches Servier.

**Poster**

486. Tau and Tauopathies
Title: Electrical and network neuronal properties are preferentially disrupted in dorsal, but not ventral, medial entorhinal cortex in a mouse model of tauopathy

Authors: *T. RIDLER*¹, C. BOOTH³, T. K. MURRAY⁴, M. A. WARD⁴, M. GOODFELLOW², K. G. PHILLIPS⁴, A. D. RANDALL¹, J. T. BROWN¹,³;
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Abstract: The entorhinal cortex provides the main interface between the hippocampus and the cortex and is one of the first areas to be affected in dementia. Neurones in the medial entorhinal cortex (mEC) display a dorsal-ventral gradient in a number of neurophysiological properties ranging from intrinsic excitability of stellate cells to grid cell firing field spacing, the functional output by which certain mEC cells respond in specific spatial locations within an environment. Here we explore the cellular and network properties of neurones in dorsal and ventral layer II/III mEC in rTg4510 mice, a transgenic model of tauopathy. Electrophysiological recordings were performed both in vitro and in vivo from 7-9 month old, male Tg4510 and wild-type (Wt) littermate control mice. Dorso-ventral gradients in certain intrinsic membrane properties such as membrane capacitance and after-hyperpolarizations are flattened in rTg4510 mEC stellate cells (SCs). Specifically, the intrinsic properties of rTg4510 mEC-SCs in dorsal aspects of the mEC are preferentially affected, such that action potential firing patterns in dorsal mEC-SCs are altered, whilst those in ventral mEC-SCs are unaffected. We also found that gamma frequency band (30-80 Hz) neuronal oscillations in vitro induced by low concentrations of kainic acid (500 nM) are preferentially disrupted in the dorsal mEC of rTg4510 slices, whilst those in ventral regions are comparatively preserved. We next determined whether these deficits were present in an intact system by chronically implanting 16-channel linear silicon probes along the dorso-ventral axis of the mEC of Tg4510 and Wt control mice. The extent of theta-modulation of gamma frequency local field potential oscillations varied along the dorso-ventral axis of the
mEC in Wt mice, such that cross-frequency coupling was higher in dorsal vs ventral electrodes. In rTg4510 mice, however, this clear gradient in theta-gamma coupling was abolished. Deficits in mEC activity may have functional implications for grid cell firing field geometry and may contribute to the impairment in spatial information processing observed in this mouse model and clinical dementia.


**Poster**

486. Tau and Tauopathies

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 486.19/C73

**Topic:** C.02. Alzheimer's Disease and Other Dementias

**Support:** ARUK-SRF2012-6

**Title:** Early stage alterations to prefrontal cortex neurophysiology in the rTg4510 transgenic mouse model of tauopathy

**Authors:** *L. E. STANIASZEK*, J. T. BROWN;

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**Abstract:** The prefrontal cortex (PFC) is critical for higher-order cognitive processes, such as the association of particular actions and locations with reward. Neurodegenerative diseases affecting the PFC, such as frontotemporal dementia can present pathologically with neurofibrillary tangles (NFTs) of the tau protein in later stages of disease progression. However, neurophysiological changes within the PFC and the timeline of these changes, remains unknown. The rTg4510 transgenic mouse expresses a mutant form of tau clinically associated with frontotemporal dementia with Parkinsonism. These mice develop age-related neuropathology (NFTs, neurodegeneration) correlated with deficits in spatial- and recognition-memory tasks. However, the effect of this form of tauopathy on cognitive tasks commonly associated with the PFC (such as working memory) is not known. In this study we sought to determine if working memory deficits could be detected at an early-stage, prior to significant neuropathology. We also used *in vivo* electrophysiology to explore global- and task-related effects of tau overexpression on PFC neurophysiology. To achieve this, we examined working memory in a delayed non-matched-to-
sample alternating T-maze task in 10 rTg4510 (TG) and 10 wildtype (WT) littermate control female mice (aged 4-5 months), over seven days. Simultaneously, we recorded PFC pre-limbic cortex (PrL) single unit action potentials (AP) and local field potentials (LFP) from the same cohort of mice. Analysis of single unit AP characteristics and the degree to which their firing was phase-locked to the LFP was undertaken to elucidate alterations at the cellular and network level in these mice. At this age point, TG and WT mice performed equally well on the behavioural task, with no statistically significant differences between the two groups at any stage of the task. Nevertheless, analysis of PrL neurophysiology illustrated that APs recorded from TG mice had a significantly wider peak-to-trough interval. Furthermore, TG neurons were less likely to fire high frequency (>67 Hz) bursts of APs than equivalent WT cells. Analysis of network characteristics revealed a trend towards increased phase-locking of action potentials to the local field-potential on correct choice trials vs force or incorrect choice trials on the first day of testing, in WT but not TG mice. These analyses suggest that in this model of dementia, changes to cellular neurophysiology in the PrL precede network and behavioural deficits.

Disclosures: L.E. Staniaszek: None. J.T. Brown: None.
cognition. We used GAL4-UAS to express the human tau protein, which is associated with neurodegenerative diseases such as Alzheimer’s disease, in circumscribed brain regions of the fruit fly. Adult females were tested in a place learning assay at multiple time points post-eclosion. Following learning trials and memory tests, flies were sacrificed and the brains were removed and the expression of tau and ptau were analyzed using immunoblot and SDS-PAGE. Flies show a decrease in learning and memory as well as a shortened lifespan and this correlates with the expression of tau in the nervous system in a time dependent manner. The phosphorylation of tau appears to also increase with time. However, the specific impact that the levels of tau vs. ptau are having on spatial cognition is unknown. It has been suggested that ptau leads to cytosolic toxicity in Drosophila, so this may be having an impact on spatial cognition. The results have allowed us to start looking at how the expression of the human tau protein in the fruit fly model is impacting learning and memory. By examining the limitations of the fruit fly model and investigating the molecular pathways and the quantitative expression levels of the disease associated protein tau in the nervous tissue of fruit flies and correlating that with a quantitative measure of spatial cognition, we can further enhance our understanding of neurodegenerative diseases in humans.


Poster

486. Tau and Tauopathies

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 486.21/C75

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Pioglitazone and memantine effects on memory impairment and tau hyperphosphorylation in intracerebroventricular-streptozotocin injected rats

Authors: *T. PONCE-LOPEZ1,2, M. ABASCAL-DÍAZ2, G. LIY-SALMERÓN2, A. MENESES1; 1CiNVESTAV, Mexico, Mexico; 2Univ. Anáhuac México Norte, Mexico City, Mexico

Abstract: The intracerebroventricular (icv) administration of the streptozotocin (STZ) has been found to induce an insulin resistance brain state (IRBS), a new, no-transgenic, animal model that has been proposed as a representative model of Alzheimer disease sporadic. Insulin could be regulating tau phosphorylation in neurons. Hyperphosphorylation of tau is considered as one of the typical pathological changes in Alzheimer Disease. During the development of AD tau is
phosphorylated at multiple sites as a result of the imbalance of numerous Ser/Thr kinases like glycogen synthase kinase 3β (GSK3β) and protein phosphatase 2A (PP2A), and integrates paired helical filament to lead to intraneuronal neurofibrillary tangles, losing their physiological functions. Previously, we demonstrated that memory deficit has been associated to increased abnormal hyperphosphorylation of tau protein, GSK3β activation and inhibition of PP2A in the hippocampus and prefrontal cortex (PFC) induced by icv administration of STZ in rats. Indicating that probably such kinase and phosphatase contribute to regulation of phosphorylation of tau. This study aims to investigate the effect of chronic treatment of pioglitazone and memantine on memory impairments, disruption insulin receptor (IR), phosphatidylinositol-3-kinase/protein kinase B/GSK3β (PI3K-Akt/PKB-GSK3β) signaling cascade, PP2A and abnormal phosphorylation of tau induced by STZ-icv. Wistar rats were bilaterally injected with STZ-icv (3 mg/kg). Memory was assessed by autoshaping, an associative learning task. The rats were administrated orally with memantine (5 mg/kg), pioglitazone (30 mg/kg) or vehicle fifty days before the last autoshaping testing test. Animals were sacrificed three months after of STZ-icv treatment, the brain were removed and dissected hippocampus and PFC. We are quantifying IR, PI3K, PKB/Akt, GSK3β, PP2A, and tau protein by Western blot. Results showed that STZ-icv treated rats had deficits in short- (1.5 h) and long-term (24 and 48 h) memory after one month and progressive memory impairment following three months of icv STZ injection relative to control rats. Pioglitazone and memantine was effective in reversing memory deficits. This data will be discussed with changes associated to hyperphosphorylation of tau, IR, key proteins downstream signaling cascade insulin and PP2A. This evidence will provide insight about the role IRBS and the therapeutic benefits of insulin sensitizer (pioglitazone) and NMDA antagonist (memantine) on abnormal phosphorylation tau and memory dysfunction.


Poster

486. Tau and Tauopathies

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 486.22/C76

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Alzheimer's Association

Cure PSP

Florida Department of Heath Ed and Ethel Moore Alzheimer's Disease Program
Examining the role of the polyamine system in animal models of tauopathy

Authors: *L. A. SANDUSKY¹, W. J. D. FRASER¹, H. SHAIR¹, A. M. BARAKAT¹, N. M. SLOUHA¹, K. RATNASAMY¹, J. B. HUNT¹, K. NASH², D. C. LEE¹


Abstract: In a non-diseased brain, the protein tau is responsible for stabilizing microtubules; however, this protein becomes hyperphosphorylated, aggregates, and promotes microtubule dysfunction eventually resulting in neuronal death in tauopathies. Polyamine dysfunction has been observed in numerous disease states, and may contribute to pathology and cognitive impairment seen in tauopathies. Our lab has recently identified polyamine dysregulation, specifically changes in spermidine/spermine N1-acetyltransferase (SAT1), spermine oxidase (SMOX) and spermine synthase (SMS), in P301L tau transgenic (rTg4510) mice. Conversely, we have shown that increasing polyamines by arginase 1 (Arg1) overexpression decreases tau neuropathology in vivo, suggesting that Arg1 and polyamines may impact tau pathology. However, a question that remains is whether these effects on tau pathology are due to the depletion of L-arginine or the production of polyamines. To answer this question and further elucidate the relationship between the polyamine system and tau biology, we examined the role of polyamine catabolic enzymes (SAT1), polyamine producing enzymes (arginine decarboxylase (ADC) and Arg1), and arginine depleting enzymes (arginine deiminase (ADI), and the impact on tau neuropathology and cognitive processing in transgenic or knockout mice. The first model examined the effect of increased polyamine production, through decreased polyamine catabolism (SAT1+/+ and SAT1-/-), using viral-mediated tau (AAV9-tauD421; C-terminally truncated at D421). The second model examined the effect of viral-mediated polyamine production and L-arginine depletion, through overexpression (AAV9-Arg1 and AAV9-ADC, or AAV9-ADI, respectively), in tau transgenic mice (MAPT P301S; PS19 model). All injections were given bilaterally to the hippocampus and anterior cortex while behavioral testing included measures of both affect and cognition. While both models produced tau-mediated cognitive impairment, the effect of arginine metabolism and/ or potentially polyamine modulation was significant both in the presence of and independent of tau. Taken together, our results identify polyamine enzymes and arginine depleting enzymes as not only being necessary for normal cognitive and affective function, but that their interaction with tau produces significant alterations in cognitive and affective processing, identifying this system as playing a key role in tauopathies. Immunohistochemical and western blotting neuropathological data will examine effects of genotype and treatments as well as correlations with behavioral performance to support and extend our findings.

Poster

486. Tau and Tauopathies

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 486.23/C77

Topic: C.02. Alzheimer’s Disease and Other Dementias

Support: Alzheimer Society of Canada

CIHR Grant MOP-102723

Title: Tau-induced down-regulation of BDNF in transgenic mouse models of tauopathy

Authors: E. ROSA¹, S. MAHENDRAM¹, Y. KE², L. ITTNER², S. D. GINSBERG³, *M. FAHNESTOCK¹;

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Abstract: In Alzheimer’s disease (AD), soluble tau is hyperphosphorylated, and some of this population presumably aggregates and precipitates as neurofibrillary tangles and/or neuropil threads. Although many theories exist, a precise toxic mechanism of tau is not well understood. We hypothesized that soluble tau-induced neurotoxicity is due to its ability to decrease trophic support for affected neurons. Specifically, our goal was to determine if over-expression of wild-type tau can down-regulate brain-derived neurotrophic factor (BDNF), a pro-survival marker that shows pathway deficits in AD and in animal and cellular models of AD. Mouse models of normal, not mutated, tau over-expression (8c-het and hTau transgenic mouse models) were used to examine the effect of excess tau on BDNF expression. 8c-het mice over-express wild-type human tau on a heterozygous mouse tau background, and while they exhibit increased tau phosphorylation and altered tau isoform expression compared to wild-type mice, they do not develop neurofibrillary tangles. On the other hand, hTau mice, which over-express wild-type human tau on a null mouse tau background, exhibit neurofibrillary tangle pathology similar to that found in human AD and tauopathies. Cortical tissue from both of these tau over-expressing models shows significantly down-regulated BDNF mRNA compared to wild-type animals, as quantified by qRT-PCR. Similarly, APP23 mice, which over-express soluble Aβ, also have significantly reduced BDNF expression. When crossed with Tau knockout (KO) mice, cortical tissue from the resulting APP23xTauKO animals exhibits BDNF expression levels intermediate between APP23 and wild-type animals and not statistically different from wild-type animals. Our
results demonstrate that excess wild-type, soluble tau can down-regulate BDNF, and that neither a mutation in tau nor neurofibrillary tangles are required for toxicity as measured by BDNF expression. Furthermore, the partial rescue of BDNF levels by tau knockout suggests that tau contributes to Aβ-induced BDNF down-regulation. Thus, loss of BDNF may mediate tau neurotoxicity, which has profound implications for therapeutic intervention in AD and tauopathies.

**Disclosures:** E. Rosa: None. S. Mahendram: None. Y. Ke: None. L. Ittner: None. S.D. Ginsberg: None. M. Fahnestock: None.

**Poster**

486. Tau and Tauopathies

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 486.24/C78

**Topic:** C.02. Alzheimer’s Disease and Other Dementias

**Support:** Anonymous

**Title:** Chronic *in vivo* imaging of tau aggregation and toxicity in the rTg4510 mouse model

**Authors:** *R. E. BENNETT, S. L. DEVOS, B. T. HYMAN; Neurol., Massachusetts Gen. Hosp., Charlestown, MA

**Abstract:** Neurofibrillary tangles (NFTs) containing tau protein aggregates are a key feature of Alzheimer’s disease. The development of NFTs is closely correlated with both the severity and duration of dementia (Arriagada 1992, Bierer 1995) as well as the amount of neuronal loss (Gomez-Isla 1997). However, NFTs are also present in cognitively normal individuals (Price and Morris 1998, Braak and Tredici 2014) and the precise relationship between the development of NFTs and toxicity is unclear. *in vivo* two-photon imaging via cranial windows has been used by our group and others to visualize protein aggregation, but longitudinal imaging has been restricted to the use of dyes and compounds that can be delivered intravenously or peripherally. Here we adapted a method for creating re-sealing silicon ports in cranial window coverglass (Roome 2014) which allows repeated topical application of dyes. We used this technique in rTg4510 mice which express mutant human tau P301L (SantaCruz 2005) to repeatedly image tau aggregation with either thioflavin S or thiazine red in tandem with Hoechst 33342 nuclear labeling to identify cell loss. Additional indicators of cellular degeneration were tested through cranial window port application including Sytox, the caspase indicating reagents NucView and FLICA, and propidium iodide. Altogether, this method allows further investigation of tau...
aggregation and toxicity in vivo over the period of weeks and months. Future experiments will use this method to deliver therapeutic agents such as antibodies via ports and image their effects over time.

Disclosures: R.E. Bennett: None. S.L. DeVos: None. B.T. Hyman: None.

Poster
486. Tau and Tauopathies

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 486.25/C79

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Suppression of mutant P301L tau in young neurons slows the course of tauopathy in the rTg4510 mouse model

Authors: *C. VOLBRACHT, P. JUL, L. HELBOE; H. Lundbeck A/S, Valby, Denmark

Abstract: In Alzheimer’s disease (AD), the microtubule associated protein tau dissociates from microtubules and forms aggregates of hyperphosphorylated tau termed neurofibrillary tangles (NFTs), which are neuropathological hallmarks of the disease. We characterized the neuropathological and behavioural consequences of a tauopathy in the rTg4510 mouse, overexpressing an inducible human mutant tau (Tau_{P301L}) selectively in the forebrain (SantaCruz et al., 2005). We observed an age-dependent progression of tau pathology including tau hyperphosphorylation and NFTs followed by late neurodegeneration in cortex and hippocampus. However, rTg4510 mice displayed early deficits in cognitive performance in the Morris water maze (MWM) already at 6 weeks of age preceding tau pathology. Continuous tau transgene suppression with doxycycline from conception was sufficient to fully prevent tau pathology, neurodegeneration and cognitive deficits. Initiation of tau suppression in adult rTg4510 mice was able to reduce tau pathology and neurodegeneration depending on the time points of intervention. Tau suppression in adult rTg4510 mice initiated at 6 weeks significantly improved learning and memory in 16-20 weeks old rTg4510 mice, but not to the levels of non-transgenic littermates. Interestingly, short term tau suppression from conception to 1 week or 3 weeks of age in young rTg4510 mice dramatically delayed appearance of tau pathology and cognitive impairment by more than 6 months. In rTg4510 mice which underwent this transient short tau suppression during development, manifestation of tau pathology and cognitive impairment coincided. These findings indicate that a critical time frame during brain development exists in the rTg4510 mouse model, which contributes essentially to the mnemonic behaviour and progression of tauopathy.
Disclosures: C. Volbracht: None. P. Jul: None. L. Helboe: None.

Poster

487. Molecular and Protein Abnormalities in Neurodegeneration

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 487.01/C80

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: KHIDI Grant HI14C1913
NRF Grant 2005-0093836
Asan Institute for Life Sciences Grant 2015-624

Title: Metallothionein-3 modulates Aβ endocytosis in astrocytes through its effect on actin polymerization

Authors: *B.-R. SEO1, S.-J. LEE1, J.-Y. KOH1,2;
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Abstract: Whereas neurons are the main cell type that is afflicted in Alzheimer’s disease (AD), astrocytes may also play important roles in amyloid beta (Aβ) metabolism such as endocytosis. Recently, we have shown that metallothionein 3 (Mt3) contributes to actin polymerization and the related signaling cascades in astrocytes. Since actin cytoskeleton is likely involved in endocytosis of Aβ, in the present study, we investigated the possible role of Mt3 in Aβ endocytosis by cortical astrocytes. Cultured cortical astrocytes were exposed to FITC-conjugated Aβ, and its endocytosis was observed under confocal fluorescence microscope. Whereas Aβ endocytosis was almost completely blocked by chloropromazine, an inhibitor of clathrin-dependent endocytosis, no change was resulted with addition of MβCD, an inhibitor of caveolin-dependent endocytosis. Clathrin-mediated endocytosis was almost completely blocked by actin disruption with CytD or LatB. Likewise, Mt3 null cells that showed defective actin polymerization, exhibited markedly reduced the clathrin-dependent CtxB uptake as well as FITC-Aβ endocytosis as compared with WT cells. In addition, Western blots showed that intracellular levels of Aβ, both monomers and oligomers, were reduced in Mt3 null astrocytes. Finally, we tested whether actin disruption or Mt3 null state changed the expression and distribution patterns of clathrin itself and the associated protein PICALM. CytD, LatB, and Mt3 null state all increased overall levels of clathrin and PICALM in astrocytes. Consistently,
immunocytochemistry showed that these conditions increased clathrin and PICALM immunoreactivities in these cells. Taken together, our results indicate that the absence of Mt3 may reduce Aβ uptake in astrocytes through the abnormality in actin polymerization. In light of evidence that Mt3 is downregulated in AD, this mechanism may contribute to the extracellular accumulation of Aβ in this disease.

**Disclosures:** B. Seo: None. S. Lee: None. J. koh: None.

**Poster**

487. Molecular and Protein Abnormalities in Neurodegeneration

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 487.02/C81

**Topic:** C.02. Alzheimer's Disease and Other Dementias

**Support:** Yonsei University University-industry Foundation Grant 2014064545

**Title:** Identification of a novel regulatory mode for Alzheimer’s disease-associated DSCR1 protein stability through USP22-mediated de-ubiquitination

**Authors:** A. HONG¹, D. KIM¹, *H. RHIM², K. C. CHUNG¹; ¹Dept. of Systems Biology, Col. f Life Sci. and Biotech., Yonsei Univ., Seoul 120-749, Korea, Republic of; ²Korea Inst. Sci. Tech. (KIST), Seoul, Korea, Republic of

**Abstract:** Protein ubiquitination can be reversed by de-ubiquitinating enzymes (DUBs). DUBs, which include ubiquitin-specific proteases (USPs) and ubiquitin C-terminal hydrolases, target several key proteins involved in regulation of tumorigenesis, apoptosis, senescence, and autophagy. DSCR1 (also known as RCAN1 or MCIP1) functions as an endogenous inhibitor of calcineurin signaling. In addition, DSCR1 modulates the balance of cell survival and cell death, and the aberrant expression of DSCR1 might be closely related to the pathogenesis of Alzheimer disease. In the present study, we have identified a novel interaction between USP22 and DSCR1 (DSCR1-1S) in the mammalian cells. In addition, the overexpression of USP22 caused the increase of DSCR1 protein stability. USP22 antagonized the actions of FBW7, NEDD4-2, and β-TrCP E3 ligase on DSCR1 and promoted DSCR1 de-ubiquitination. Moreover, we found that DSCR1 was bound to USP22 in basal conditions, and IFN-α treatment caused the dissociation of DSCR1 from USP22, which subsequently triggered DSCR1 ubiquitination and proteasome degradation. Taken together, these results suggest that USP22 positively regulates DSCR1 levels, which would consequently affect diverse DSCR1-linked cellular processes, such as the inflammatory process involving the release of IFN-α.

Poster

487. Molecular and Protein Abnormalities in Neurodegeneration

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 487.03/C82

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: PO1AG14449

RO1AG043375

P30AG010161

Title: Epigenetic and endosomal-lysosomal dysfunction in the basal forebrain during the progression of Alzheimer’s disease

Authors: *L. MAHADY;
Barrow Neurolog. Inst., Phoenix, AZ

Abstract: Epigenetic and endosomal-lysosomal dysfunction in the basal forebrain during the progression of Alzheimer’s disease *L. Mahady1,2, M. Nadeem1, B. He1, S.E. Perez1, E.J. Mufson1 1Dept. of Neurobiology, Barrow Neurological Institute, Phoenix, AZ 85013, 2Dept. Neurological Sci., Rush University, Chicago, IL and 3Arizona State University Interdisciplinary Graduate Program in Neuroscience, Tempe, AZ Basal forebrain neuronal degeneration occurs during the progression of Alzheimer’s disease (AD). However, the factors underlying the onset of basal forebrain cellular dysfunction remains unclear. Recent findings indicate that endosomal-lysosomal (E-L)/autophagic dysregulation occur prior to β-amyloid (Aβ) plaque and tau tangle pathology and may play a role in neuronal selective vulnerability during the progression of AD. However, E-L alterations likely act in concert with other factors to drive neuronal degeneration. In fact, epigenetic factors regulate E-L gene transcription and function. For example, histone deacetylases (HDAC6 and HDAC2) and methylated histone H3 lysine 9 (H3K9) are implicated in the pathogenesis of AD. Whether E-L/autophagic and epigenetic changes co-occur in the basal forebrain during the onset of AD remains unknown. Here we quantified changes in the E-L/autophagic proteins cathepsin D (Cat D), rab5, respectively, and the epigenetic markers HDAC2, HDAC6, and H3K9 using frozen basal forebrain tissue obtained from subjects who died with a premortem clinical diagnosis of NCI (n=7; mean age=87; mean MMSE=28), MCI (n=7; mean age=91; mean MMSE=24), mild/moderate AD (n=8; mean age=90; mean
MMSE=20) and severe AD (n=8; mean age=75; mean MMSE=6.7) from the Rush Religious Orders Study (RROS) and the Rush RADC, respectively. Groups were matched by age and postmortem interval (PMI=5 hr) and underwent detailed postmortem neuropathologic evaluations. Western blot analysis of tissue homogenates revealed stable levels of HDAC2 and HDAC6 across the four clinical groups examined. Increased levels of dimethylated H3K9, Cat D, and rab5 were found in severe AD compared to NCI, MCI and mild/moderate AD. We also observed a strong positive correlation between both H3K9 and Cat D (r=0.73), and H3K9 and rab5 (r=0.65), suggesting that epigenetic factors play a role in the regulation of E-L systems in AD. In summary, these results indicate that the basal forebrain is resilient to E-L disturbances early in the disease process. Since, E-L and epigenetic dysregulation occur late in the disease process, this molecular interaction may exacerbate neuronal degeneration in severe AD.

Disclosures: L. Mahady: None.

Poster

487. Molecular and Protein Abnormalities in Neurodegeneration

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 487.04/C83

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: FFG Grant 844453

Title: Alzheimer’s disease-related vascular pathology in human and transgenic mouse brain

Authors: M. TEMMEL1, *J. NEDDENS1, D. HAVAS1, C. SCHWEINZER1, J. ATTEMS2, H. HUTTER3, B. HUTTER-PAIER1;
1QPS Austria Gmbh, Grambach, Austria; 2Inst. for Ageing and Health, Univ. of Newcastle, Newcastle upon Tyne, United Kingdom; 3Inst. of Cell Biology, Histology and Embryology, Med. Univ. of Graz, Graz, Austria

Abstract: During recent years evidence for a connection between neurovascular dysfunction and the pathogenesis of Alzheimer’s disease (AD) has accumulated. A vascular factor, specifically the ε4 allele of APOE, is so far the only common accepted genetic risk factor for AD beside the imperative effects of APP and Presenilin mutations. Alterations in the brain vasculature, such as microvascular atrophy, decreased clearance of amyloid β (Aβ), and loss of astrocytic water channels, come more and more into focus as probable upstream events. The current study was therefore designed to investigate whether vascular changes associated with human AD may be replicated in the transgenic APPSLL mouse model. Using indirect immunofluorescence and
quantitative image analysis, AD-associated vascular changes in cortical areas of healthy and diseased human brain sections and in both transgenic APPSL mice and non-transgenic controls were investigated. We found that the occurrence and progression of cerebral amyloid angiopathy (CAA) is associated with age in APPSL mice, whereas a positive correlation of CAA and Braak stage was less obvious in human AD. The approach was then extended to test AD-related hypotheses on collagen IV, and the data show total intensity of collagen IV immunofluorescence increases similarly in the hippocampus of transgenic mice and in AD individuals. Detailed analysis revealed that in mice this is due to enlargement of existing blood vessels, whereas in human subjects it is associated with an increasing numerical density of blood vessels. Astrocytes express aquaporin 4 (AQP4), a water channel enabling active water transport. The evaluation suggests that AQP4 is expressed following complex spatio-temporal patterns in humans and transgenic mice. An abnormally low amount and a delocalization of hippocampal AQP4 were detected at early Braak stage. If confirmed by additional investigations, this could be potentially useful as an early marker for AD. In conclusion, the present study supports the notion that the brain vasculature is affected in AD and that APPSL mice represent a useful model for studying different aspects of blood vessel-associated AD-related histopathology.


Poster

487. Molecular and Protein Abnormalities in Neurodegeneration

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 487.05/C84

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant AG031388

Title: Shankopathis in Alzheimer’s disease

Authors: *Y. GONG*\(^1\,^2\), F. E. CHOW\(^2\), R. M. TSAI\(^2\), C. F. LIPPA\(^2\);
\(^1\)Nanjing Univ. Chinese Med., Jingsu, China; \(^2\)Dept. of Neurol., Drexel Univ. Col. of Med., Philadelphia, PA

Abstract: Alzheimer’s disease (AD) is a progressive neurodegenerative disorder characterized by synaptic loss and cognitive degeneration. Synaptic loss correlates strongly with disease severity but the underlying mechanism remains elusive. During the pathological progression of AD, Aβ oligomers induce the disruption of glutamate receptors in the macromolecules of the
Shank-network located in the postsynaptic density (PSD). Shank proteins show distinct pathological changes which contribute to N-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor loss at the PSD in AD. The oligomers of Aβ can induce hyperphosphorylation in tau and neurofilament (NF) proteins in AD, which may cause the same pathological changes in the Shank proteins. Dephosphorylation in hyperphosphorylated tau proteins is dependent on the activity of peptidyl-prolyl cis/trans-isomerase Pin1 and protein phosphatases. During this investigation, we found the Pin1 colocalized with Shank3 and the loss of Pin1 activity altered the macromolecules of the Shank-network by ubiquitin proteasome system at the PSD, thus contributing to the synaptic dysfunction and loss in the early stages of AD. This suggest that the loss of Pin1 activity may be the common initial pathological change leading to the extreme modifications in synaptic proteins as observed in amyloid precursor protein (APP) and Tau in preclinical AD. Shank proteins could play a pathological role in the cognitive deficiency in AD.


Poster

487. Molecular and Protein Abnormalities in Neurodegeneration

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 487.06/C85

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Brinson Foundation (SEP)

Title: Hippocampal cathepsin D and p62 association with APP/Aβ processing and tau pathology during the progression of AD

Authors: *S. E. PEREZ¹, H. CHoudary¹, E. J. Mufson²;

Abstract: Hippocampal endosomal-lysosomal (E-L) and autophagy dysregulation may underlie neuronal selective vulnerability during the progression of Alzheimer’s disease (AD). Our previous western-blot finding 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 mild/moderate AD, but not changes occurred in autophagy markers. Since E-L pathways and autophagy are major routes of amyloid precursor protein (APP) processing and tau dysregulation, we examined whether hippocampal intraneuronal Cat D was associated with intraneuronal APP/Aβ and neurofibrillary tangle (NFT)
(phosphorylated AT8 tau) pathology, endosomal (rab5 and rabaptin5) and the autophagy marker (p62), a mTOR upstream signaling protein involved in cargo recognition and autophagosome formation that plays a critical role in autophagy failure and is associated to NFT formation early in AD. Hippocampal neuronal Cat D and intraneuronal APP/Aβ levels were quantified using immunohistochemistry and densitometry in paraffin sections from subjects who died with a premortem clinical diagnosis of non-cognitive impairment (NCI), MCI or mild to moderate AD obtained from the Rush Religious Study (ROS) cohort. Adjacent hippocampal sections were immunolabeled with antibodies against p62. Optical density (OD) analysis revealed no changes in neuronal Cat D- and APP/Aβ (6E10)-immunoreactive (-ir) levels within the different hippocampal subfields. In addition, the density p62-ir neurons were unchanged in the different hippocampal layers across the clinical groups examined. Hippocampal neuronal Cat D OD values correlate positively with intraneuronal APP/Aβ OD values in CA1 pyramidal neurons during AD progression (r=0.660, p=0.001). Conversely, OD measurements of Cat D-ir neurons were not associated with rab 5, rabaptin5 or neurofibrillary AT8 and p62 neuronal density. Interestingly, p62 and AT8-ir neuronal density showed a strong relationship within all hippocampal subfields across the groups examined (p<0.0001). These data reinforce and extend our previous observations that Cat D alterations in hippocampal neurons occur late in AD and may be associated to APP/Aβ processing rather than tau pathology, while the association between hippocampal AT8 and p62 neuronal density indicates a major role of autophagy in NFT formation.

Disclosures: S.E. Perez: None. H. Choudary: None. E.J. Mufson: None.

Poster

487. Molecular and Protein Abnormalities in Neurodegeneration

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 487.07/C86

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Grant-in-aid for Scientific Research (C) 25460343

Title: An Alzheimer's disease-linked mutant T835M-UNC5C causes neuronal cell death by activating an intracellular death signal cascade

Authors: *Y. HASHIMOTO, M. MATSUOKA;
Tokyo Med. Univ., Tokyo, Japan
Abstract: A previous study showed that a missense mutation (T835M) in the UNC5C gene appears to cause autosomal-dominant late-onset Alzheimer’s disease in two families and that various insults lead to increased death in neurons expressing T835M-UNC5C. In this study, we found that overexpression of T835M-UNC5C itself causes prominent death while overexpression of wild-type UNC5C causes minimal death in F11 neurohybrid cells and SH-SY5Y cells. Netrin inhibits this death. T835M-UNC5C-induced neuronal cell death is mediated by an intracellular death signal pathway consisting of DAPK1/PKD/ASK1/JNK/caspases. These results may provide a new insight on the pathomechanism of Alzheimer’s disease.

Disclosures: Y. Hashimoto: None. M. Matsuoka: None.

Poster

487. Molecular and Protein Abnormalities in Neurodegeneration

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 487.08/C87

Topic: C.02. Alzheimer’s Disease and Other Dementias

Support: NIH NINDS R01NS081208-01A

Title: Transcriptomics profiling of Alzheimer’s disease reveal neurovascular defects, altered amyloid β homeostasis and deregulated expression of long noncoding RNAs

Authors: *M. MAGISTRI, D. VELMESHEV, M. MAKHMUTOVA, M. FAGHIHI; Psychiatry, Univ. of Miami, Miami, FL

Abstract: Background: The underlying genetic variations of late onset Alzheimer’s Disease (LOAD) cases remain largely unknown. A combination of genetic variations with variable penetrance and lifetime epigenetic factors may converge on transcriptomics alterations that drive LOAD pathological process. Transcriptome profiling using deep sequencing technology offers insight into common altered pathways regardless of underpinning genetic or epigenetic factors and thus represents an ideal tool to investigate molecular mechanisms related to the pathophysiology of LOAD. Results: We performed directional RNA sequencing on high quality RNA samples extracted from hippocampi of 4 LOAD and 4 age-matched controls and we further validated our data using qRT-PCR on a larger set of post-mortem brain tissues. Pathway analysis indicates dysregulation in neural communication, cerebral vasculature and Amyloid β clearance. Beside protein coding genes, we identified several annotated and non-annotated long noncoding RNAs that are differentially expressed in LOAD brain tissues, three of them are activity-dependent regulated and one is induced by Aβ1-42 exposure of human neural cells. Conclusions:
Our data provide a comprehensive list of transcriptomics alterations in LOAD hippocampi and warrant holistic approach including both coding and non-coding RNAs in functional studies aimed to understand the pathophysiology of LOAD.


Poster

487. Molecular and Protein Abnormalities in Neurodegeneration

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 487.09/C88

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Sie foundation postdoctoral award

Linda Crnic Institute seed Grant

MNIRGDP-12-258900

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RO1-NS086933

NARSAD 21069

Title: Role of the RCAN1 isoforms on calcineurin activity and mitochondrial morphology

Authors: *C. A. ZAMBRANO; H. WONG; S. KIM; E. ZIFF; C. HOEFFER;[1,2,5];


Abstract: Regulator of calcineurin 1 (RCAN1) protein is an important modulator of Ser/Thr phosphatase, calcineurin (CaN). Three isoforms of RCAN1 are produced in the brain, RCAN 1.1L, RCAN 1.1S and RCAN 1.4. RCAN1 overexpression has been linked to oxidative stress and mitochondrial dysfunction and is also elevated in sporadic Alzheimer’s disease (AD). RCAN1 overexpression may be involved in the progression of Alzheimer’s disease by regulating CaN activity related to mitochondrial function. One potential substrate for RCAN1/CaN regulation activity related to AD-associated mitochondrial dysfunction is dynamin-related protein 1 (DRP1). We have recently shown that, DRP1 phosphorylation is modulated by overexpression of the RCAN1.1S isoform during aging. In this study, we show that all RCAN1
isoforms modulate CaN activity using a fluorescence resonance energy transfer (FRET) based CaN activity reporter 1(CANAR1) approach. To do this, RCAN1 isoforms were transfected in both HEK393 human cell lines and mouse primary hippocampal neuronal cultures. CANAR1 was co-expressed with RCAN isoforms to measure CaN activity. RCAN1.1S and RCAN1.4 isoforms were able to modulate calcineurin activity in a cell specific fashion, observing an increase in activity in primary neurons but the opposite effect in HEK cells. This result highlights the importance of the context of where RCAN1 is being study. In genetically modified cre-dependent mouse primary neurons that overexpress RCAN1.1S isoform (RCANtg) it was possible to confirm the positive regulation of that particular isoform on CaN activity. RCANtg neurons display smaller mitochondria than control neurons, suggesting a role of RCAN1.1S on mitochondrial morphology. Moreover, RCANtg neurons have elevated oxidative stress relative to control cre(-) neurons determined with CellROX® fluorescence assay. These results reinforce the idea of RCAN1.1S isoform as a key component of the neurodegenerative process observed in Alzheimer’s pathology.

Disclosures: C.A. Zambrano: None. H. Wong: None. S. Kim: None. E. Ziff: None. C. Hoeffer: None.

Poster

487. Molecular and Protein Abnormalities in Neurodegeneration

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 487.10/C89

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant K99NS087096

NIH Grant DK31405

NIH Grant DK90861

Title: Bri2, a gene mutated in Alzheimer-like dementias, is a functional partner of irisin

Authors: *C. D. WRANN*¹,², K. GERBER¹,², M. JEDRYCHOWSKI³, L. YANG²,³, V. MOOTHA²,³, M. SCHUMACHER⁴, L. D’ADAMIO⁵, H. TU⁶, S. GYGI², B. SPIEGELMAN¹,²; ¹Dana-Farber Cancer Inst., Boston, MA; ²Harvard Med. Sch., Boston, MA; ³Massachusetts Gen. Hosp., Boston, MA; ⁴Duke Med. Sch., Boston, MA; ⁵Albert Einstein Col. of Med., New York, NY; ⁶Lakepharma Inc., Belmont, CA
Abstract: Exercise can improve cognitive function and the outcome of neurodegenerative diseases, like Alzheimer’s disease. Recently, we have reported a FNDC5/irisin pathway which is activated by exercise in the hippocampus in mice and induces a neuroprotective gene program. Affinity-purification of irisin complexes from the media of primary cortical neurons followed mass spectrometry identifies the transmembrane protein ITM2b as a binding partner of irisin. ITM2b, also known as Bri2, is the causal gene mutated in two forms of Alzheimer-like dementias in humans, British Familial Dementia and Danish Familial Dementia. Co-immunoprecipitation experiments with recombinant proteins confirmed the interaction. Bioinformatical analysis using the CLIME algorithm indicates that FNDC5 and ITM2B are members of same strongly evolutionary conserved cluster. Bri2 positively regulates the protein levels of FNDC5 and its secreted form irisin, without changing Fndc5 mRNA expression. Interestingly, in primary cortical neurons from Bri2−/− mice overexpression of FNDC5 full-length protein or stimulation with irisin recombinant protein fails to induce the neuroprotective gene program compared to neurons from Bri2+/+ mice. Taken together, this indicates a functional relationship between irisin and Bri2, a gene mutated in Alzheimer-like dementias. This work was supported by the JPB This work was supported by the JPB Foundation and NIH grants (DK31405 and DK90861) and a Pathway to Independence (PI) Award (K99NS087096) to C.D.W.


Poster

487. Molecular and Protein Abnormalities in Neurodegeneration

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#Poster#: 487.11/C90

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Intraneuronal Abeta accumulation increases vulnerability to oxygen-glucose deprivation and excitotoxicity

Authors: *L. CALZA1,2, V. A. BALDASSARRO1, A. MARCHESINI2, M. FERNÁNDEZ1, L. GIARDINO1,2; 1CIRI-SDV, Univ. of Bologna, Ozzano Emilia, Italy; 2IRET Fndn., Ozzano Emilia (BO), Italy

Abstract: Microvascular dysfunction is considered an integral part of Alzheimer disease (AD) pathogenesis. Several working hypothesis attempt to clarify the possible relationship between
amyloid pathology, microvascular dysfunction and cell death. In fact, Abeta fragments inhibit angiogenesis, interact with VEGF receptors and alter glutamatergic transmission, interacting with NMDA receptors and glutamate uptake and release. Moreover, APP expression is increased following ischemic or hypoxic conditions, stimulating the production and deposition of Abeta peptide in the brain. In order to investigate the influence of intraneuronal Abeta accumulation on vulnerability to glutamate excitotoxicity and Oxygen Glucose Deprivation (OGD), primary cortical neurons isolated from Tg2576 (carriyng the hSwAPP-mutation) and WT new-born mice were used as in vitro model, and exposed to glutamate (42µM; 10 minutes) or OGD (3 hours, followed by 24 hours reoxigenation). Cell death (nuclear morphology) and human APP levels (immunocytochemistry using the 6E10 antibody) were analysed using cell-based High Content Screening. VEGF and VEGF receptors (Flt1 and Kdr) mRNA expression was analysed by using real time PCR. Neurons isolated from Tg2576 new-born mice showed an increase in VEGF mRNA expression (2.47 times, p<0.05) and a decrease in the expression of the two VEGF receptors (Flt1: 0.56 times, p<0.001; Kdr: 0.31 times p<0.001), compared to WT cells. Tg2576 primary neurons displayed higher spontaneous cell death (WT: 13.93%; Tg2576: 26.24% p<0.001) and the percentage of pyknotic/fragmented nuclei is higher in Tg2576 cells (60.02%) compared to WT (54.16%; p<0.001) when cultures were exposed to glutamate. When exposed to OGD, Tg2576 neurons show higher cell death in terms of percentage of pyknotic/fragmented nuclei (WT: 22.40%; Tg2576: 29.37% p<0.05) and mitochondrial depolarization (percentage of cells showing low intensity of the mitochondrial dye MitoTracker; WT: 31.53%; Tg2576: 44.49%, p<0.001). When exposed to OGD Tg2576 primary neurons show an increase in the 6E10 immunoreactivity intensity (Tg2576 OGD 14.6% of increase p<0.001). This study showed that the presence of the mutated human APP gene, leading to the intracellular accumulation of APP and Abeta fragments, alters the sensitivity of cortical neurons to glutamate excitotoxicity and OGD. In a mutual correlation, the expression pattern of genes related to OGD response is modified in cells carrying the transgene, and the APP accumulation is worsened by OGD.


Poster

487. Molecular and Protein Abnormalities in Neurodegeneration

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 487.12/C91

Topic: C.02. Alzheimer’s Disease and Other Dementias
Support: DFG, SFB 877
       DFG, GRK 1459

Title: ADAM10-mediated shedding significantly impacts on prion disease

Authors: *M. GLATZEL*1, B. PUIG2, P. SAFTIG3, H. C. ALTMEPPEN2;
1Inst. of Neuropathology, Univ. of Hamburg, Hamburg, Germany; 2Christian Albrechts Universit, Christian Albrechts University, Kiel, Germany

Abstract: Proteolysis of adhesion molecules, growth factors and receptors play key roles in neurological diseases. In neurodegenerative diseases such as prion disease or Alzheimer’s disease, proteolytic processing of proteins critically influences disease initiation and propagation. For prion diseases, α-cleavage of the cellular prion protein (PrPC) impairs misfolding into the pathogenic isoform (PrPSc) and is thus protective. The role of PrP-shedding (a physiological cleavage event occurring in close proximity to the GPI-anchor of PrPC) remained largely unknown. We and others have identified ADAM10 as the physiologically relevant sheddase of PrPC regulating its membrane homeostasis. Here we show that depletion of ADAM10 in forebrain neurons leads to posttranslational increase of PrPC levels. When infected with prions, these mice present with drastically shortened incubation times, increased PrPSc formation and upregulation of calpain. Our spatiotemporal analyses also suggest that absence of shedding impairs spread of prion pathology within the brain. Taken together, ADAM10-mediated shedding seems to have a dual role in prion diseases thus emphasizing the relevance of proteolytic processing in prion disease. Given the suggested role of PrPC as a receptor for toxic protein oligomers in more common proteinopathies our findings might impact on these devastating conditions as well.

Disclosures: M. Glatzel: None. B. Puig: None. P. Saftig: None. H.C. Altmeppen: None.

Poster

487. Molecular and Protein Abnormalities in Neurodegeneration

Location: Hall A

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Program#/Poster#: 487.13/C92

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant F30 NS090893-01 (WAM)
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NIH Grant RO1AG033004 (JYW)
ALS Therapy Alliance Grant (JYW)

Title: Fus-regulated microRNAs in fus proteinopathy

Authors: *W. A. MCGEE* 1,2,3, J. DENG 4,5,6, Y. FU 7, H. CHENG 1,2,3, K. FUSHIMI 1,2,3, X. CHEN 1,2,3, S. KUROSAKA 8, T. TAKUMI 8, A. XU 7, J. Y. WU 1,2,3;
1Neurol., 2Ctr. for Genet. Med., 3Lurie Cancer Ctr., Northwestern Univ. Feinberg Sch. of Medicin, Chicago, IL; 4State Key Lab. of Biocontrol, 5Natl. Engin. Ctr. of South China Sea for Marine Biotech., 6Dept. of Biochem., Sun Yat-sen Univ., Guangzhou, China; 7Inst. of Biophysics, Chinese Acad. of Sci., Beijing, China; 8RIKEN Brain Sci. Inst., Wako, Japan

Abstract: Fused in Sarcoma / Translocated in Sarcoma (FUS/TLS, or FUS) is a multifunctional DNA/RNA binding protein known to be involved in diverse processes of gene regulation, including DNA repair, transcription, pre-mRNA splicing, RNA transport, RNA stability and translation. Since 2009, it has been known that intracellular inclusion bodies containing FUS are the pathological hallmark of a subset of cases of Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal Lobar Degeneration (FTLD). These cases have since been reclassified as FUS proteinopathy. Despite much recent efforts to elucidate the pathogenesis of FUS proteinopathy, much remains unknown about the biological function of FUS in the nervous system and the role(s) of FUS in disease pathogenesis. Emerging evidence from our data and others suggests a previously unknown role of FUS in microRNA biogenesis. MicroRNAs play important roles in a wide range of biological processes. Aberrant regulation of microRNA genes contributes to human diseases, including various aging related disorders such as neurodegeneration and cancer. Here, we systematically examined miRNAs whose expression levels are regulated by FUS using paired expression profiles of miRNAs and mRNAs using small-RNA-Seq and mRNA-Seq, respectively. We compared the brains of control and FUS-deficient mice. We have also begun to examine and compare the post-mortem brain tissue from well-characterized FTLD-FUS proteinopathy patients and control subjects. Using these paired expression profiles, we developed a computational pipeline to identify candidate microRNAs regulated by FUS, examine the sets of genes targeted by each candidate miRNA, and predict which microRNA-mRNA gene networks are altered in the FUS-deficient brain samples. Specifically, we used miRExpress and eXpress to map the reads to the genome, Probabilistic miRNA-mRNA Interaction Signature (ProMIne) to predict miRNA-mRNA interactions for each sample, and a combination of DESeq and Fatiscan to examine the sets of genes for each candidate miRNA and their associated processes. At the time of submission, we completed the computational analysis, with several microRNAs identified as candidates and major signaling pathways identified related to synaptic signaling and mitochondrial function. We have begun to validate these computational predictions using molecular cellular, biochemical and cell biological approaches. The computational pipeline, previously unknown miRNA-target gene pairs that may play important roles in FUS
proteinopathy, and new contributions to our understanding of the role of FUS in pathogenesis of neurodegenerative disorders will be discussed.


Poster

487. Molecular and Protein Abnormalities in Neurodegeneration

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 487.14/C93

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Consortium for Frontotemporal Dementia Research

NIH Grant T32HD071866

American Federation for Aging Research Glenn/AFAR Postdoctoral Fellowship

Title: Aav-mediated overexpression of progranulin corrects social behavior deficits in grn+/- mice

Authors: *A. E. ARRANT, E. D. ROBERSON; Neurol., Univ. of Alabama At Birmingham, Birmingham, AL

Abstract: Loss-of-function mutations in progranulin (GRN), a secreted glycoprotein with neurotrophic and anti-inflammatory effects in the brain, are a major cause of frontotemporal dementia (FTD), accounting for 5-10% of all FTD cases. Boosting progranulin levels is therefore a promising strategy for preventing or treating FTD-GRN. This approach is supported by data from cultured neurons, but has not been tested in an animal model. In this study, we tested the benefits of increasing progranulin levels with an AAV vector in Grn+/- mice, a mouse model of FTD due to GRN mutations. Grn+/- mice develop reduced social dominance in the tube test and reduced sociability in the three-chamber sociability test around 9 months of age. We injected an AAV 2/1 virus expressing progranulin with a C-terminal myc tag (AAV-Grn) or a control AAV-Gfp virus into the medial prefrontal cortex (mPFC) of 10-13 month-old wild-type and Grn+/- mice to determine if AAV-Grn could correct these social phenotypes. AAV-Grn treatment boosted progranulin levels to approximately 7-fold over wild-type levels in the mPFC, and significantly increased progranulin levels in the septum and medial areas of the dorsal and ventral striatum. Four to six weeks after AAV injection, AAV-Grn-treated Grn+/- mice were more dominant in the tube test and spent more time interacting with a novel mouse in the three-
chamber sociability test than did AAV-Gfp-treated Grn+/− mice. These data support progranulin-boosting therapies for FTD-GRN by providing, to our knowledge, the first data showing that increasing progranulin levels corrects an FTD-like phenotype in a mouse model of FTD-GRN.

**Disclosures:** A.E. Arrant: None. E.D. Roberson: None.

**Poster**

**487. Molecular and Protein Abnormalities in Neurodegeneration**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 487.15/C94

**Topic:** C.02. Alzheimer's Disease and Other Dementias

**Support:** Lundbeck Foundation, grant no. R167-2013-15940

**Title:** Endosomal pathway deficiencies in fibroblasts from FTD-3 patients

**Authors:** *N. ROSTGAARD*1, J. E. NIELSEN2, T. T. NIELSEN2; 26991, 1Danish Dementia Res. Centre, Univ. Hospit, Copenhagen OE, Denmark

**Abstract:** Frontotemporal dementia constitutes one third of dementia cases and a small percentage of these are caused by autosomal dominant mutations in a few identified genes. Frontotemporal dementia linked to chromosome 3 (FTD-3) is a rare familial dementia, described in a large Danish family, caused by a single base mutation in the *CHMP2B* gene leading to early-onset dementia. *CHMP2B* encodes a component of the ESCRTIII-complex which is located in the membrane of endosomes. The complex is necessary for the formation of multivesicular bodies (MVBs) - a late endosomal compartment. Previous studies have shown an enlarged endosomal phenotype in patient brains, recapitulated in patient fibroblasts and transgenic mice. Furthermore deficiencies in endosomal trafficking and autophagy have been shown in FTD-3 fibroblasts and several cell models transfected with the CHMP2B mutation. Autophagy and lysosomal membrane permeabilization (LMP) contributes to degenerative diseases but the role of LMP in neurodegeneration is poorly understood but could contribute to disease pathogenesis in diseases were the endocytic pathway is either disrupted or malfunctioning as in FTD-3. Here we investigate the cellular disease pathology and assess endosomal pathway, autophagy deficiencies and LMP in fibroblasts from symptomatic and presymptomatic mutation carriers using immune staining and confocal microscopy. Further we evaluate the gene expression of several genes with key roles in the pathways affected by the CHMP2B mutation by Q-PCR such as vesicular transport, autophagy and Ca2+ signaling.

Poster

487. Molecular and Protein Abnormalities in Neurodegeneration

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 487.16/C95

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: National Institute of Neurological Disorder and Stroke (NS085770)

The Louis Family Foundation

The Davee Foundation of Neurobiology Research Initiative Fund

Northwestern University Alzheimer's Disease Center (AG013854)

Title: Time-dependent formation and disappearance of TDP-43 inclusions in a conditional transgenic mouse model of FTLD

Authors: *L. KUKREJA*, G. KIM¹, K. SADLEIR², L. WANG³, H. DONG³, J. CSERNANSKY⁴, M.-M. MESULAM¹, R. VASSAR², C. GEULA¹;


Abstract: Dementias caused by Frontotemporal lobar degeneration (FTLD) constitute the third most prevalent dementia, after those caused by Alzheimer’s disease and Lewy bodies, and are among the most prevalent dementias of early-onset. The vast majority of these cases contain abnormal precipitates of a phosphorylated and mislocalized form of the TAR DNA/RNA-binding protein-43 (TDP-43). Moreover, overexpressing wild-type or mutant human TDP-43 gene in transgenic animals results in the formation of inclusions and neuronal loss, which have led to the conclusion that TDP-43 pathology leads to FTLD. To directly investigate the temporal sequence of the appearance of TDP-43 inclusions and its relationship to pathology, we employed a conditional transgenic mouse line in which expression of wild-type human TDP-43 is under the control of tetracycline operator sequences. In this study, transgene expression was switched off from birth until weaning age by doxycycline treatment in the mouse diet in order to avoid previously reported complex phenotypes of early neuronal development. In accordance with previous findings, the induction of human TDP-43 recapitulated features of FTLD-TDP, including the formation of phospho-TDP-43 neuronal cytoplasmic inclusions and progressive neurodegeneration. Our immunohistochemical analyses using an antibody that recognizes TDP-
43 phosphorylated at Ser-403/404 revealed that inclusions appear as early as 5 days following TDP43 transgene expression. Mice which express the transgene for 10 days show a moderate density of inclusions. The inclusions appear to peak by 14 to 19 days post-transgene expression and decline rapidly thereafter. At these early days of TDP-43 transgene expression, the inclusions are present across frontal, parietal, and temporal cortical areas, and the hippocampus. While inclusions were absent at 8 weeks and 24 weeks of TDP-43 transgene expression, qualitative analysis showed severe neuronal loss in the dentate gyrus. However, the dentate gyrus contained among the lowest densities of inclusions. Thus, the density of TDP-43 inclusions does not directly correlate with neuronal loss in this animal model. It is likely that intracytoplasmic accumulation of TDP-43 oligomers plays a more direct role in neuronal loss and perhaps explains neurodegeneration in the absence of inclusions. Our findings suggest that this TDP-43 mouse model might provide critical information towards understanding how TDP-43 aggregation is linked to neurodegeneration and behavioral deficits in FTLD.


**Poster**

**487. Molecular and Protein Abnormalities in Neurodegeneration**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 487.17/C96

**Topic:** C.05. Aging

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  European Regional Development Fund.

**Title:** Neuroinflammaging impairs the mechanisms of response against an acute inflammatory injury: a lipopolysaccharide study in the mouse model of neurodegeneration SAMP8

**Authors:** C. SANFELIU1, P. MOLINA-MARTÍNEZ1, R. CORPAS1, P. KALIMAN1, M. COSÍN-TOMÁS2, R. CRISTÓFOL1, C. SOLÀ1, *G. MENGOD3, M. PALLÀS4, J. L. MOLINUEVO3, A. LLADÓ5; 1IIBB-CSIC, IDIBAPS, Barcelona, Spain; 2IIBB-CSIC, UB, Barcelona, Spain; 3IIBB-CSIC, IDIBAPS, CIBERNED, Barcelona, Spain; 4UB, CIBERNED, Barcelona, Spain; 5Alzheimer's Dis. and other Cognitive Disorders Unit, HCB, IDIBAPS, Barcelona, Spain
Abstract: Inflammaging in the elderly is a risk factor for age-related diseases that share an inflammatory component, including neurodegenerative diseases. Moreover, the resultant chronic low grade neuroinflammation is increasingly considered a significant actor in the triggering and progression of Alzheimer’s disease (AD). The senescence accelerated prone mouse P8 (SAMP8) is a model of pathological aging and AD, showing a maximum lifespan of shortly 18 months, cognitive loss, increased levels of amyloid and hyperphosphorylated tau, oxidative stress and neuroinflammation. We aimed to study the progression of brain inflammatory derangement in SAMP8 by testing basal status and response to an injection of lipopolysaccharide (LPS) at the early age of 6 months and at the advanced stage of pathological changes of 12 months. The level of the proinflammatory cytokines IL6, IL1β and TNFα was determined by ELISA in blood and cerebral cortex, whereas their gene expression was determined by quantitative RT-PCR in cerebral cortex and hippocampus. Brain tissue of 6-month old SAMP8 showed higher inflammatory markers than control mice SAMR1. Moreover, LPS induced an inflammatory response in both strains, although SAMP8 showed a potentiation of the response as compared to SAMR1. Strikingly, 12-month-old SAMP8 showed lower inflammatory markers and lack of activation by LPS, whereas the response was preserved in SAMR1 mice. Therefore, SAMP8 cerebral inflammatory mechanisms were overreactive at 6 months of age and nearly nonresponsive to stimulation at 12 months of age. Studies in mixed glial culture and in pure microglia obtained from newborn SAMP8 and SAMR1 strains, showed higher levels of cytokine release to the culture medium in response to LPS/interferon γ (IFN) in SAMP8 than SAMR1 cultures. Furthermore, the measure of nitrites accumulated in the culture medium indicated higher activation of NO production in both SAMP8 cell type cultures after LPS/INF stimulation, but also in SAMP8 microglia in basal conditions. Therefore, chronic inflammation in the brain of SAMP8 mice relays in early hyperactivated glia. Chronic inflammation would let to an impaired response to external proinflammatory stimulus in the aged SAMP8 brain. Therefore, neuroinflammaging greatly contributes to disruption of neural defense mechanisms against homeostatic disturbances. This loss of brain physiological reserve will pave the way to frailty and AD. Acknowledgements: Grants CSD2010-00045 and SAF2012-39852 from the Spanish MINECO, and the European Regional Development Fund.


Poster

487. Molecular and Protein Abnormalities in Neurodegeneration

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM
Program/#Poster#: 487.18/D1

**Topic:** C.02. Alzheimer's Disease and Other Dementias

**Support:** National Institute of Neurological Disorders and Stroke (NS085770)

Northwestern University Alzheimer's Disease Center (AG013854)

**Title:** Asymmetric distribution of activated microglia in a left-handed patient with primary progressive aphasia, TDP-43 pathology and right hemisphere language dominance

**Authors:** G. KIM¹, S. VAHEDI¹, S. WEINTRAUB¹, *C.-K. WU², E. BIGIO¹, M.-M. MESULAM¹, C. GEULA¹;
¹Cognitive Neurol. and Alzheimer's Dis. Ctr., Northwestern University, Feinberg Sch. of Med., Chicago, IL; ²Neurol., Texas Tech. Univ. Sch. of Med., Lubbock, TX

**Abstract:** Primary progressive aphasia (PPA) is a neurodegenerative clinical dementia syndrome characterized by language deficits as the most salient clinical feature and atrophy in the perisylvian language network in the dominant hemisphere (usually the left). The asymmetric nature of the language network and focal atrophy render PPA an excellent model for investigation of the relationships between the regional distribution of pathologic markers, cortical atrophy and clinical phenotype. The subject in this study was a left-handed patient with PPA in whom the language network was lateralized to the right hemisphere as indicated by functional MRI before death. In a preliminary stereological analysis we had observed significantly high densities of TDP-43 inclusions in the superior temporal gyrus (STG) and the inferior temporal gyrus (ITG), with asymmetry favoring the right hemisphere, matching the patterns of atrophy as seen by structural MRI. The next highest density of TDP-43 inclusions was observed in other language-related cortical areas such as inferior frontal gyrus (IFG), inferior parietal lobule (IPL) and middle frontal gyrus (MFG, area 9), but without hemispheric asymmetry. The lowest density of TDP-43 inclusions was detected in the memory related area entorhinal cortex (ERC). In this study, an antibody to HLA-DR was used to obtain measures of activated microglia bilaterally, and to determine concordance with TDP-43 inclusion density and cortical atrophy. Unbiased stereological techniques were used to quantify activated microglia in IFG, MFG, IPL, STG, ITG, and ERC. Activated microglia were found in relatively high density in all cortical areas, with slightly lower density in ERC. Significantly, microglial density displayed substantial asymmetry favoring the right hemisphere in all cortical areas. However, this asymmetry was greatest in STG and ITG, matching the asymmetry in TDP-43 inclusions. These findings suggest that microglial activation does not share a linear relationship with the density of TDP-43 inclusions. However, the two pathological markers share a pattern of greatest asymmetric distribution in the same cortical areas and display concordance with the PPA clinical phenotype and patterns of atrophy.
**Disclosures:**  
**G. Kim:** A. Employment/Salary (full or part-time); Northwestern University.  
**S. Vahedi:** A. Employment/Salary (full or part-time); Rosalind Franklin University of Medicine and Science.  
**S. Weintraub:** A. Employment/Salary (full or part-time); Northwestern University.  
**C. Wu:** None.  
**E. Bigio:** A. Employment/Salary (full or part-time); Northwestern University.  
**M. Mesulam:** A. Employment/Salary (full or part-time); Northwestern University.  
**C. Geula:** A. Employment/Salary (full or part-time); Northwestern University.  

**Poster**  
**487. Molecular and Protein Abnormalities in Neurodegeneration**  
**Location:** Hall A  
**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM  
**Program#/Poster#:** 487.19/D2  
**Topic:** C.02. Alzheimer’s Disease and Other Dementias  
**Support:** Frederick J. Pelda Alzheimer’s Research Fund  
**Title:** Determining the role of TDP-43 in Alzheimer’s disease-related neurodegeneration  
**Authors:**  
*K. D. LACLAIR*¹, P. C. WONG²;  
¹Cell. and Mol. Med., ²Pathology, Neurosci., Johns Hopkins Sch. of Med., Baltimore, MD  

**Abstract:** The development of disease modifying treatments for Alzheimer’s disease (AD) has been severely limited by poor understanding of its molecular determinants and under-appreciation for its underlying mechanistic diversity. Indeed, recent studies show that most AD cases exhibit various additional pathologies other than the canonical amyloid-β and tau aggregates. One of these non-canonical pathologies is TDP-43 proteinopathy occurring in 30-50% of cases, characterized by cytoplasmic aggregation of TDP-43 accompanied by its nuclear clearance. Independent studies also showed that TDP-43 proteinopathy in AD correlates strongly with worse cognition, greater neurodegeneration, and increased severity of tau pathology. Tdp-43 depletion in mice leads to age-dependent neurodegeneration, supporting the view that loss of nuclear TDP-43 could contribute to disease pathogenesis. However, it is unclear whether TDP-43 depletion and β-amyloidosis have distinct or interconnected roles in neurodegeneration, and whether loss of function in TDP-43 affects β-amyloid production or deposition. To address these critical questions, we created a mouse line with age-dependent β-amyloidosis and conditional loss of Tdp-43 in mature forebrain neurons. We assess β-amyloid production and pathology, neurodegeneration, and frank neuron loss in these mice compared to those with only β-amyloidosis or forebrain depletion of Tdp-43. These studies begin to clarify how TDP-43 proteinopathy affects neurodegeneration in the context of β-amyloidosis, and may lead to the identification of therapeutic targets for the treatment of this large sub-population of AD patients.
Disclosures: K.D. Laclair: None. P.C. Wong: None.

Poster

487. Molecular and Protein Abnormalities in Neurodegeneration

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 487.20/D3

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NSC 102-2320-B-006-040-MY3

MOST 103-2321-B-006-028

Title: Autophagy activation is a new light for treatment of TDP-43 proteinopathies from Drosophila to mammalian FTLD-U and ALS disease models

Authors: C.-W. CHENG1,2, I.-F. WANG1,2, *K.-J. J. TSAI3, C.-K. SHEN1;

Abstract: TDP-43 is a multi-functional DNA/RNA-binding protein that has been identified as the major component of the cytoplasmic inclusions in the diseased cells of frontotemporal lobar dementia (FTLD) and amyotrophic lateral sclerosis (ALS). Unfortunately, effective drugs for these neurodegenerative diseases are yet to be developed. We have tested the therapeutic potential of rapamycin, an inhibitor of the mammalian target of rapamycin (mTOR), in a FTLD-U mouse model with TDP-43 proteinopathies as well as ALS-TDP fly model. We showed that rapamycin administration at the early pathological stage of a mouse model with FTLD characterized with cytoplasmic TARDBP/TDP-43(+) inclusions in the diseased neurons could rescue the learning/memory deficiency and the abnormal motor function disorder of the mice. Moreover, autophagy activation at a late pathological stage also could improve motor function, which was accompanied by a reduction of the TARDBP(+) inclusions. In ALS-TDP flies, autophagy activation also could rescue partially locomotor activity and lifespan. These studies have set the principal for therapy of neurodegenerative diseases with the TARDBP protein, i.e., ALS-TDP and FTLD-TDP, with the use of autophagy activators. Key word: TDP-43 proteinopathies, ALS, FTLD, rapamycin, autophagy

Poster

488. Network Oscillations in Parkinson's Disease: Human Studies

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 488.01/D4

Topic: C.03. Parkinson’s Disease

Support: National Institute for Health Research University College London Hospitals Biomedical Research Centre

MRC UK Clinical Research Training Fellowship MR/K022172/1

MRC/EPSRC UK MEG Partnership award

The Wellcome Trust 091593/Z/10/Z

Title: Deep Brain Stimulation in Parkinson’s disease reduces cortico-subthalamic oscillatory synchrony

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Abstract: Deep Brain Stimulation of the subthalamic nucleus (STN) is an effective treatment for Parkinson’s Disease (PD), yet its mechanisms of action and influences on cortico-STN networks are unknown. It has previously been shown that two spatially and spectrally distinct resting STN-cortical networks exist in PD: 1) An STN-temporo-parietal alpha band (7-12 Hz) network and 2) An STN-premotor/motor beta (13-30 Hz) band network. We sought to examine the effects of DBS on these networks. We performed simultaneous magnetoencephalography (MEG) and bilateral STN local field potential recordings (LFPs) in 15 post-operative PD patients, whose DBS electrodes had been temporarily externalised. Using a purpose built amplifier we were able to stimulate the STN and record its activity during concurrent MEG recording. Cortical sources coherent with the STN were reconstructed using beamforming. Monopolar DBS in the MEG scanner generated severe artefacts rendering some MEG channels unusable. However, we used a phantom recording to show that physiological brain activity could still be recovered under these conditions. Analysis of patient data showed that unilateral monopolar DBS at 130 Hz suppressed low beta power (11-14 Hz) locally within the STN (p< 0.05) in addition to suppressing coupling between the STN and mesial premotor areas (including supplementary motor area, SMA) across the entire beta (15-30 Hz) frequency range (p <0.01). In contrast, alpha band coupling with the
temporal cortex was not significantly altered by DBS. DBS-related clinical motor improvements, assessed through part III of the unified PD rating scale score, correlated with reductions in local beta power in the STN across subjects ($r^2 = 0.38$, $p < 0.01$), but not with the reductions in STN-mesial premotor coupling. We show that MEG can be used to study the effect of DBS on oscillatory cortico-subcortical networks. Our results confirm previous findings suggesting that DBS may exert therapeutic benefit through suppression of low beta activity locally within the STN. Furthermore, we found a suppression of broad beta band coupling between the STN and mesial premotor structures which could be indicative of exaggerated activity in the hyperdirect pathway in PD.

**Disclosures:** V. Litvak: None. A. Oswal: None. M. Beudel: None. A. Jha: None. T. Foltynie: 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.; "Exenatide as a treatment for Parkinson's disease" - Michael J fox Foundation. D. Fees for Non-CME Services Received Directly from Commercial Interest or their Agents (e.g., speakers' bureaus); Medtronic Inc., St Jude Medical. F. Consulting Fees (e.g., advisory boards); Abbvie Pharmaceuticals. P. Limousin: D. Fees for Non-CME Services Received Directly from Commercial Interest or their Agents (e.g., speakers’ bureaus); Medtronic Inc., St Jude Medical. F. Consulting Fees (e.g., advisory boards); Abbvie Pharmaceuticals. L. Zrinzo: D. Fees for Non-CME Services Received Directly from Commercial Interest or their Agents (e.g., speakers’ bureaus); Medtronic Inc., St Jude Medical. M.I. Hariz: D. Fees for Non-CME Services Received Directly from Commercial Interest or their Agents (e.g., speakers’ bureaus); Medtronic Inc., St Jude Medical. P. Brown: F. Consulting Fees (e.g., advisory boards); Medtronic Inc.

**Poster**

**488. Network Oscillations in Parkinson's Disease: Human Studies**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 488.02/D5

**Topic:** C.03. Parkinson’s Disease

**Title:** Amplified movement-related cortical desynchronization and decoupling allow normal motor responses in patients with Parkinson’s disease and essential tremor

**Authors:** *E. D. KONDYLIS, M. J. RANDAZZO, A. ALHOURANI, W. J. LIPSKI, T. A. WOZNY, A. S. GHUMAN, M. RICHARDSON, D. J. CRAMMOND; Dept. of Neurosurg., Univ. of Pittsburgh, Pittsburgh, PA
Abstract: Alpha (8-12) and beta (12-35 Hz) rhythms are thought to function in sensorimotor cortices to maintain the motor status quo, since movement related desynchronization (MRD) in these frequency bands accompanies motor execution. In patients with Parkinson’s disease (PD), synchronous cortical beta activity has been shown to be more widespread, more stable, and more tightly coupled with high frequency activity (phase-amplitude coupling, PAC), compared to subjects with dystonia or without a movement disorder. Importantly, these relationships are modulated by therapeutic deep brain stimulation of the subthalamic nucleus, suggesting that cortical beta activity is important in maintaining the hypokinetic symptoms of PD. The role of synchronous cortical activity in ET is less well studied, though modulation of alpha and beta band activity with therapeutic thalamic DBS has been documented. Significant gaps remain, however, in our understanding of how the timing and spatial distribution of MRD and PAC are altered in patients with movement disorders. The objective of this study was to examine the cortical dynamics of MRD in patients undergoing deep brain stimulation (DBS) for PD and ET, and in patients without a movement disorder undergoing intracranial monitoring for seizure mapping. We applied a monetarily incentivized bimanual grip force task to 27 subjects (12 PD, 9 ET, 6 epilepsy) while recording local field potentials (LFP) from subdural electrodes temporarily implanted over sensorimotor cortex, including hand primary motor cortex. Patients with PD and ET exhibited increased alpha and beta synchrony in sensorimotor cortex in the absence of movement, compared to patients without a movement disorder. We found that successful movement was accompanied by a greater extent of MRD, in both movement disorder groups. A reduction with movement in alpha and beta synchrony and beta-gamma PAC in hand sensorimotor and premotor cortices was common to all three groups. Beta MRD predominated in PD while alpha MRD predominated in ET, and both groups exhibited significantly greater MRD than subjects without a movement disorder (p <10^{-14}, p<10^{-7}). In addition, movement-related reductions in PAC also were significantly greater in PD and ET (p < 10^{-2}, p < 10^{-2}), with alpha-gamma decoupling predominating in ET and beta-gamma decoupling predominating in PD. These results demonstrate that patients with PD and ET can produce vigorous movement, but that they must overcome a larger burden of cortical synchrony and coupling. This is the first study to demonstrate a common neural mechanism through which patients with different movement disorders can achieve similar execution of motor actions.


Poster

488. Network Oscillations in Parkinson's Disease: Human Studies

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM
Title: Phase-amplitude coupling between beta band and high-frequency oscillations as a marker for motor impairment in Parkinson’s disease

Authors: *B. C. M. VAN WIJK¹, M. BEUDEL², A. JHA³, A. OSWAL¹, T. FOLTYNIE², P. LIMOUSIN², L. ZRINZO³, M. I. HARIZ³, P. BROWN³, V. LITVAK¹;

Abstract: Exaggerated beta band oscillations within the subthalamic nucleus are a hallmark of Parkinson’s disease. However, it remains unclear how this leads to motor impairment. We studied cross-frequency coupling between the phase of beta band activity and the amplitude of high-frequency oscillations (around 300Hz) known to increase in strength during movement. Local field potential recordings from 33 patients who underwent bilateral implantation of deep brain stimulation electrodes in the subthalamic nucleus were analyzed. Recordings took place both after overnight withdrawal of dopaminergic medication and following levodopa administration. There was a significant positive correlation between the strength of phase-amplitude coupling and clinical scores of contralateral hemibody bradykinesia and rigidity (r=.26, p=.01). In addition, more severe motor impairments were associated with lower beta band frequencies at which phase-amplitude coupling occurred (r=.42, p<.001). Whereas phase-amplitude coupling within the subthalamic nucleus was strongest for low-beta frequencies (around 17Hz), beta band coherence with the motor cortex dominated at high-beta frequencies (around 25Hz), as determined via simultaneous magneto-encephalography. These findings support previous suggestions of a functional subdivision within the beta frequency band. Results suggest that exaggerated low beta band oscillations may hamper normal motor functioning by pathologically constraining the pro-kinetic high-frequency oscillations in the subthalamic nucleus.

Disclosures: B.C.M. Van Wijk: None. M. Beudel: None. A. Jha: None. A. Oswal: None. T. Foltynie: 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.; Michael J Fox Foundation. D. Fees for Non-CME Services Received Directly from Commercial Interest or their Agents
(e.g., speakers’ bureaus); Medtronic Inc., St Jude Medical. **P. Limousin:** D. Fees for Non-CME Services Received Directly from Commercial Interest or their Agents (e.g., speakers’ bureaus); Medtronic Inc., St Jude Medical. **L. Zrinzo:** D. Fees for Non-CME Services Received Directly from Commercial Interest or their Agents (e.g., speakers’ bureaus); Medtronic Inc., St Jude Medical. **M.I. Hariz:** D. Fees for Non-CME Services Received Directly from Commercial Interest or their Agents (e.g., speakers’ bureaus); Medtronic Inc., St Jude Medical. **P. Brown:** F. Consulting Fees (e.g., advisory boards); Medtronic Inc.

**V. Litvak:** None.

**Poster**

**488. Network Oscillations in Parkinson's Disease: Human Studies**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 488.04/D7

**Topic:** C.03. Parkinson’s Disease

**Support:** Mc Donnell Foundation

- National Parkinson's Foundation
- NIH Grant NS054864
- NIH Grant P01 NS083514

**Title:** Homeostatic regulation of beta power with motor practice is present in normal subjects but not in patients with Parkinson's disease

**Authors:** *C. MOISELLO*¹, A. B. NELSON¹, D. BLANCO¹, P. PANDAY¹, J. LIN¹, A. DI ROCCO², M. GHILARDI¹;

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**Abstract:** Parkinson’s disease (PD) is characterized by impairments in cortical plasticity, in beta power at rest and its modulation during movement. Recent results with protocols inducing long-term potentiation (LTP) in normal subjects further suggest the connection between cortical plasticity and changes of beta power recorded with EEG during rest. Here, we determined whether beta power at rest and its modulation during movement change in subjects with PD and controls with extended, repetitive practice in a simple motor task, and whether such changes are renormalized by a night of sleep. We recorded high-density EEG in 17 patients with PD and 15 age-matched controls before, during and after a 40-minute task requiring reaching movements to visual targets presented in an unpredictable order. The same task in the same conditions was performed in the morning of the next day after a night of sleep. We found that beta power at rest was significantly increased in PD patients compared to the controls. This change was preserved during practice in controls and renormalized by sleep. In contrast, beta power at rest did not change during practice in PD patients, and there was no renormalization by sleep. These findings suggest that beta power at rest and its modulation during movement are impaired in PD, and that sleep may play a role in recovery of this function.
repeated the following day. For both days, we determined post-task changes of beta power at rest and assessed the progressive changes in beta modulation during the task over a frontal and two sensorimotor regions, which showed the strongest movement-related beta modulation. In controls, we found that, on day 1, in electrodes over these three regions, a significant increase in beta power at rest was present in the spontaneous EEG at rest compared to baseline and that movement-related beta modulation increased significantly with practice. On day 2, both beta power at rest and its modulation during movement returned to the baseline values of day 1 and then increased similarly to the previous day. In patients with PD, on both day 1 and 2, beta power at rest was higher than in controls and practice-induced changes at rest, like those during movements, were markedly reduced compared to the controls. In both groups, kinematic characteristics improved with practice. We conclude that, in normal subjects, prolonged practice in a motor task produces use-dependent modifications that are reflected in changes of beta power at rest and during movement and that renormalize with rest or sleep. In PD, such changes are significantly reduced; this might represent, at least partially, impairment of cortical plasticity. The significant post-task beta increase in controls can be interpreted as a reduction of cortical excitability resulting from protracted use, a phenomenon akin to the ‘occlusion’ of LTP-like plasticity, which can be overcome by rest or sleep that restore beta levels to the original levels. The failure in patients to display changes in beta power could be ascribed to an already ‘over-inhibited’ state or an occlusion of LTP-like plasticity, and thus to a failure of homeostatic mechanisms.


Poster

488. Network Oscillations in Parkinson's Disease: Human Studies

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 488.05/D8

Topic: C.03. Parkinson’s Disease

Support: NINDS 1R01NS090913-01

Title: Dyskinesia occurring with by dopaminergic medication or DBS are Associated with a narrowband high Frequency oscillation in human chronic cortical and subcortical recordings

Authors: *N. C. SWANN*, C. DE HEMPTINNE1, S. MIOCINOVIC2, S. QASIM1, S. WANG2, N. ZIMA2, J. OSTREM2, M. SAN LUCIANO2, N. GALIFIANAKIS2, P. STARR1; 1Neurolog. Surgery, 2Neurol., Univ. of California, San Francisco, San Francisco, CA
Abstract: Introduction: Parkinson’s Disease (PD) is characterized by abnormal oscillatory activity throughout basal ganglia-thalamocortical motor loops, that is modulated by medication and deep brain stimulation (DBS). To study these patterns over time we utilized a novel, fully implantable, device, which records and stores field potentials, in addition to delivering therapeutic DBS. This method alleviates the challenges associated with an intraoperative environment and acute brain changes related to the surgical procedure. One goal of this study is to characterize electrophysiological signatures associated with adverse effects induced by medication or stimulation (such as involuntary hyperkinetic movements, known as dyskinesia.)

Methods: Five advanced PD patients were implanted with the recording/stimulating device. Each of these patients were studied at multiple visits from the time of surgery up to a year post-operatively during periods on and off medication, on and off DBS, and with and without dyskinesia. Each patient has a unilateral 4 contact electrode implanted in the subthalamic nucleus (STN) which has the capability of either stimulating or recording, and a 4 contact electrocorticography (ECoG) strip over primary motor cortex (M1) for recording only. Recordings, sampled at 800 Hz, were initiated and downloaded by radiotelemetry.

Results: We observed an increase in narrowband activity during periods of dyskinesias associated with the medication, DBS, or both. This activity is > 60 Hz and is characterized by a large increase in power in M1, and a smaller increase in STN power at the same frequency. There is also strong phase coherence between the two. The frequency at which the activity occurred was usually about 70-75 Hz when no DBS was delivered, and moved to half the stimulation frequency during DBS. This activity is generally not present during recordings without dyskinesia.

Discussion: We characterized a reliable biomarker of dyskinesia in humans: a high frequency, narrowband increase in oscillatory activity, which is especially strong in the cortex. A similar observation has been made in a rodent model of dyskinesia. We propose that therapeutic high frequency STN DBS entrains neuronal populations at a sub-harmonic of the stimulation frequency, and that this sub-harmonic drives dyskinesia. These results suggest that monitoring for this narrowband increase in activity could improve DBS delivery, by serving as a signal for closed loop control of DBS. This finding also suggests changes in STN DBS stimulation protocols may help to avoid dyskinetic adverse effects.


Poster

488. Network Oscillations in Parkinson's Disease: Human Studies

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM
Abstract: Studies have shown that the basal ganglia play an important role in beat perception, and that patients with Parkinson's disease (PD) are impaired in the perception of beat-based rhythms. This contrasts with considerable evidence for a beneficial effect of rhythmic external cues in gait rehabilitation, and raises the question how rhythm can improve movement in PD. We addressed this question with analyses of slow oscillations and beta oscillatory activity (13-30 Hz), recorded using magnetoencephalography (MEG) during a choice response task with rhythmic and non-rhythmic modes of stimulus presentation. The study comprised 15 PD patients, of mild to moderate disease severity, and 15 control subjects. Analyses focussed on (i) entrainment of slow oscillations in the delta frequency-band, (ii) the depth of beta power modulation (defined as the difference in power between maximal beta synchronization and desynchronization), and (iii) whether a gain in modulation depth of beta power, due to rhythmicity, is of predictive or reactive nature. Replicating earlier work, the results show weaker entrainment of slow oscillations, and a relative shift from predictive to reactive movement-related beta suppression in PD patients. Nonetheless, rhythmic stimulus presentation increased the beta modulation depth to the same extent in PD patients and controls. Critically, in both groups, this gain selectively increased the predictive and not the reactive movement-related beta power suppression. Together, the results indicate that PD patients and controls exploit rhythmicity in the same way, providing no support for the popular view that rhythmic stimulation confers a special advantage to PD patients. The results provide converging evidence for a recent proposal that post-movement beta synchronisation constitutes a signal involved in trial-to-trial updating of an internal model guiding future movement.


Poster

488. Network Oscillations in Parkinson's Disease: Human Studies

Location: Hall A
Title: The subthalamic nucleus processes emotion: electrophysiological differences between Parkinson’s disease and Obsessive-Compulsive Disorder patients

Authors: A. BUOT¹, *B. LAU¹, M.-L. WELTER¹,², J. YELNIK¹, E. BARDINET¹,³, S. FERNANDEZ-VIDAL¹,³, C. KARACHI¹,², L. MALLET¹,⁴; ¹Inst. du cerveau et de la moelle épinière, Paris, France; ²Assistance Publique-Hopitaux de Paris, Paris, France; ³Ctr. de Neuroimagerie de recherche, Paris, France; ⁴Personalized Neurol. & Psychiatry Univ. Dept., Hôpitaux Universitaires Henri Mondor – Albert Chenevier, Créteil, France

Abstract: The subthalamic nucleus (STN) of the basal ganglia is divided into motor, associative and limbic territories based on anatomical connectivity. The posterior and dorsolateral (motor) territory is routinely targeted to treat Parkinson’s Disease (PD) using deep brain stimulation (DBS), while the anterior and ventromedial (limbic) territory has been recently targeted to successfully treat severe refractory obsessive-compulsive disorder (OCD). To better understand how the STN processes emotional information, we recorded STN local field potentials (LFP) post-operatively in 7 PD and 4 OCD patients who underwent DBS implantation while they performed an emotional categorization task. The task consisted of the presentation of emotional stimuli (pleasant, unpleasant or neutral pictures from the IAPS), followed by a motor response in some trials. This allowed us to compare emotional and motor-related activity across two different STN territories. In all patients, stimulus presentation induced a synchronization of activity (ERS: event-related synchronization) in the theta band (3-8 Hz). This ERS was significantly stronger and lateralized (left>right hemisphere) in OCD compared to PD. The magnitude of the theta ERS varied according to stimulus valence. In PD, both ON and OFF dopaminergic medication, the theta ERS was significantly stronger for unpleasant compared to neutral stimuli, although we did not observe any effect for pleasant compared to neutral stimuli. By contrast, in OCD patients, we observed a change in theta activity for both valences, with a significantly stronger ERS for pleasant compared to unpleasant stimuli. During the motor response in PD patients, we observed a desynchronization (ERD) in the beta band (14-30 Hz) that commenced at stimulus presentation. The beta ERD magnitude depended on motor execution, dopaminergic treatment and reaction time. However, there was no significant dependence on emotional valence in the beta band. By contrast, the beta ERD was almost nonexistent in OCD patients. Our results show that STN activity varies according to location within the nucleus, with strong emotion-related
and weak motor-related modulations of oscillatory activity in the anterior and ventromedial compared to the posterior and dorsolateral STN. The striking difference in STN activity for pleasant stimuli between PD and OCD patients may be specific to the pathologies studied.


Poster

488. Network Oscillations in Parkinson's Disease: Human Studies

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 488.08/D11

Topic: C.03. Parkinson's Disease

Support: IZKF 255

NIH Grant P01 NS 083514

Title: Beta oscillations and the generation of reaching movements: lateralization of sub-cortical contribution

Authors: *A. CANESSA1,2, C. MOISELLO1, G. ARNULFO1,2, F. STEIGERWALD2, N. G. POZZI2, M. M. REICH2, M. M. FATO1, M. F. GHILARDI1, J. VOLKMAN2, I. U. ISAIAS5; 1DIBRIS - Deparment of Informatics, Bioengineering, Robotics, Syst. Engin., Univ. of Genoa, Genova, Italy; 2Dept. of Neurol., Univ. Hosp. and Julius-Maximilian-University, Würzburg, Germany; 3Dept. of Physiology, Pharmacol. and Neurosci., CUNY Med. Sch., New York, NY

Abstract: Extensive work with electroencephalographic (EEG) scalp recordings has shown that power in the beta range (13-30 Hz) is modulated during passive, active, observed or imagined movement. Studies in patients with Parkinson's Disease (PD) with Deep Brain Stimulation (DBS) have further explored the oscillatory beta activity of the subthalamic nucleus (STN). Both at cortical and subcortical level, beta power starts decreasing before movement, reaches floor values during movement execution and finally increases again after movement end. However, despite the growing body of evidence, very few studies have determined whether the two hemispheres equally contribute to the production of movement performed with the dominant and non-dominant arm. Therefore, we recorded simultaneously high-density EEG and STN local field potentials in six right-handed patients with PD, who had undergone bilateral STN electrode implantation for DBS (Activa PC+S®, Medtronic Inc., Minneapolis, USA). The patients were tested in two successive days in meds-OFF state (overnight withdrawal of all dopaminergic
drugs). They performed a motor task requiring reaching movements to visual targets presented in an unpredictable order every three seconds. On each day, they performed 50 movements both with the right and left hand. We measured several characteristics of the motor performance, including reaction time, movement speed and spatial error. There was no difference in any of the kinematic indices between the movements performed with the right and left. We also found that the depth of beta modulation was similar in the electrodes over the left and right sensori-motor cortices independently of the execution side. On the other hand, subcortical activation showed an asymmetry of modulation depth: specifically, in the right STN, beta modulation was larger for contralateral movements (i.e. performed with the left hand, day 1: \( p<0.0001 \); day 2: \( p=0.0052 \)), while the left STN showed a similar activation for movements irrespective of active hand. This is the first evidence of a differential involvement of cortical and subcortical beta rhythms in the production of dominant and non-dominant upper limb movements.


**Poster**

488. Network Oscillations in Parkinson's Disease: Human Studies

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program/Poster#:** 488.09/D12

**Topic:** C.03. Parkinson’s Disease

**Support:** NSF IGERT DGE-1069104

King Fahd Medical City Intramural Research Funds

**Title:** Cortical phase-amplitude coupling in Parkinson’s disease using magnetoencephalography

**Authors:** *E. PEÑA*¹, L. ROSEDAHL¹, M. D. JOHNSON¹, J. BAJWA²;
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**Abstract:** Phase amplitude coupling (PAC) describes the degree to which the amplitude of oscillatory activity in a high frequency band phase-locks to oscillatory activity in a lower frequency band. Such coupling is thought to be indicative of information transfer between neuronal populations at different spatiotemporal scales, and has been shown to play a critical role in cognitive processes such as learning. In some cases this type of coupling may be pathological,
and recent intraoperative studies in Parkinson’s Disease (PD) patients have noted strong coupling between gamma/high-frequency oscillatory activity and beta-band oscillations in primary motor cortex, subthalamic nucleus, and between both structures. Here, we investigate the heterogeneity of cortical PAC in human PD across a range of clinical severities, motor subtypes, and responsiveness to dopaminergic replacement therapy. In a cohort of 18 patients (5 females and 13 males; ages 55-70, disease duration of 2-9 years) and 10 age-matched healthy controls, we recorded resting-state magnetoencephalography (MEG, Elekta Neuromag) off and on medication. PAC comodulograms were constructed (phase frequency range: 1-30Hz, amplitude frequency range: 50-280Hz) based on source-space estimations of oscillatory activity in three gyri (precentral, postcentral, superior frontal) and two sulci (central, superior frontal) from both hemispheres. Significant beta-gamma PACs that were reduced by medication were found in 7 patients (2 female, 5 males). All seven patients who showed significant PAC also demonstrated symptoms of akinesia/rigidity, though two patients had predominantly tremor symptoms on one side. None of the 10 control subjects exhibited significant PAC over the sensorimotor cortex. These results demonstrate the utility of MEG to non-invasively assess cortical PACs in PD patients. In addition, the results support the multi-dimensional relationship between the clinical features of PD and cortical PACs.

Disclosures: E. Peña: None. L. Rosedahl: None. M.D. Johnson: None. J. Bajwa: None.

Poster

488. Network Oscillations in Parkinson's Disease: Human Studies

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 488.10/D13

Topic: C.03. Parkinson’s Disease

Support: NSCF Grant 81171061

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Title: Thalamic oscillatory activity in patients with Parkinsonian tremor and essential tremor

Authors: *P. ZHUANG¹, M. HALLETT², T. LIU¹, Y. ZHANG¹, J. LI¹, Y. LI¹;
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Abstract: OBJECTIVE: To characterize oscillatory activity in the ventrolateral thalamus in patients with parkinsonian tremor and essential tremor (ET). BACKGROUND: The mechanisms
of parkinsonian tremor and ET remain unclear. Parkinsonian tremor has been supposed to be associated with the basal ganglia-thalamocortical circuit while ET has been predicted to be related to cerebellar-thalamocortical circuit. Stimulation or lesioning of the ventrolateral thalamus, in particular, the ventral intermediate nucleus (Vim) can effectively abolish both parkinsonian tremor and ET suggesting that the region is likely involved in the pathophysiology of tremor.

METHODS: 26 patients with PD and 14 patients with ET who underwent thalamic surgery were studied. Microelectrode recordings in the ventral oral posterior (Vop)/Vim and EMG of contralateral limbs were performed. Single unit analysis and interspike interval (ISI) histograms were performed to assess neuronal mean firing rate (MSFR) and pattern. Spectral characteristics were evaluated. Coherence analysis was used to explore the relationship between oscillatory activity and EMG. Results: Of 196 neurons obtained from PD patients, 63.7% neurons were oscillatory. Of these, 74.4% neurons (n=93) with tremor frequency band (TFB) oscillation at mean frequency of 4.5 ± 0.9 Hz were correlated with tremor; 25.6% neurons (n=32) with β frequency band (βFB) oscillation had mean firing rate of 19.6 ± 8.9 Hz. Of 70 neurons obtained from ET, 65.7% neurons were oscillatory. Of these, 89.1% (n=41) neurons had TFB oscillation at mean frequency of 6.8 ± 4.5 Hz frequently correlated with postural tremor; 10.9% neurons (n=5) had βFB oscillation at mean of 22.2 ± 6.2 Hz. Further analysis found that the proportion of βFB oscillatory neurons in PD were significantly higher than that of βFB oscillatory neurons in ET (p<0.05). Moreover, MSFR of oscillatory neurons in PD were significantly lower than that of oscillatory neurons in ET (26.7 ±6.8 Hz vs. 42.3 ±15.6 Hz, p<0.05). There were no significant differences of proportion of TFB oscillatory neurons between PD and ET. CONCLUSIONS: The proportion of thalamic βFB oscillatory neurons is higher in PD supporting that βFB oscillatory neurons are associated with dopaminergic deficits in the basal ganglia circuit. Compared with normal MSFR (estimated to be 30 Hz from recordings in normal monkeys), the MSFR of oscillatory neurons in the VL are likely high in ET suggesting an etiologic role of the thalamus in ET.

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Poster

488. Network Oscillations in Parkinson's Disease: Human Studies

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 488.11/D14

Topic: C.03. Parkinson’s Disease

Support: CIHR MOP 98006
Title: The modulation of subcortical beta oscillations during motor learning in essential tremor and Parkinson's disease

Authors: *D. BASHA*¹², S. ELLIS⁴¹, S. KALIA⁴³, M. HODAIE⁴³, A. M. LOZANO⁴, W. D. HUTCHISON²¹⁴;
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Abstract: Beta oscillations are commonly observed in the major motor structures of the brain, including the basal ganglia, motor cortex, thalamus and cerebellum. In healthy subjects, the power of beta oscillations is suppressed during movement execution but is high during rest and postural maintenance. Recent studies have shown that the degree of this movement-related beta suppression in the motor cortex correlates positively with improved performance during motor learning. We hypothesized that beta suppression in subcortical structures is also a marker for motor learning in Parkinson’s disease (PD) patients. Beta power was measured from spike and local field potential recordings of the motor thalamus and subthalamic nucleus during microelectrode-guided deep brain stimulation surgery as patients learned a visuomotor adaptation task. Four patients performed a centre-out task (20 trials) which involved moving a cursor on a computer screen from a central starting point to equidistant targets to the left or right for the baseline condition. The display was then inverted so that leftward movements produced rightward deflections of the cursor on the screen and vice versa for the experimental condition (20 trials). In the baseline condition, mean centre-out response time improved from 6.68±4.64 seconds to 4.12±0.96 seconds (first 10 versus last 10 trials, t-test, p=0.001). In the inverted condition, mean response time improved from 7.09±4.35 seconds to 6.23±2.76 seconds (t-test, p=0.02). Trial by trial analysis revealed that movement-related beta suppression was highest in trials where response time was shortest. These preliminary findings suggest that beta oscillations correlate with improved motor performance during motor learning.


Poster

488. Network Oscillations in Parkinson's Disease: Human Studies

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 488.12/D15

Topic: C.03. Parkinson’s Disease
Support: CIHR MOP 98006  
DMRF

**Title:** Microstimulation-induced tremor oscillations in movement disorder patients

**Authors:**  
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**Abstract:** Tremor at rest affects approximately 75% of patients suffering from Parkinson’s disease (PD) but is not a cardinal symptom of dystonia. Theories of tremorgenesis seem to fall into a “thalamocentric” view (Pare et al 1992) and a “pallidocentric” view (Helmich et al. 2011). Evidence for each theory is hampered by the lack of demonstration of pacemakers; cells or circuits that are capable of oscillating at the tremor frequency in the absence of sensory feedback, i.e. central pattern generators. Here we present evidence that neurons with the internal segment of the globus pallidus (GPI) of dystonia patients initiate a sustained tremor frequency oscillation following recovery from microstimulation-induced inhibition. Two independently driven microelectrodes were advanced through the GPI of PD and dystonia patients undergoing DBS implantation surgery. Upon isolation of single, stable recording units, 10s of baseline neuronal activity was recorded followed by a brief microstimulation (3/5/7.5μA, 1s duration, 0.3ms biphasic pulse width, 200Hz). Cells were then recorded for 10s following stimulation and traces were saved for offline analysis in Spike2 software. Traces were bandpass filtered (300-3000Hz) before being template matched to generate spike times. These times were then imported into MatLab for spike pattern and burst analysis with an in-house script. A total of 95 neurons from 9 PD (n =58) and 7 dystonia (n= 37) patients were analyzed of which, 19 were identified as border cells. For PD cells stimulated at 3 μA no overall change was seen, 73 Hz to 72 Hz (n= 23, NSD) 5 μA decreased from 80 Hz to 66 Hz (n = 50, p < 0.05) and at 7.5μA, 85Hz to 58 Hz (n= 15, p< 0.05). Burst indices at 3 μA did not change (2.03 to 2.01,) and at 5 μA increased (1.8 to 2.5) and 7.5 μA (1.7 to 2.5). Six neurons defined as border cells were induced into a tremor frequency oscillation post stimulation (4 - 6 Hz). Border cells have previously not been found to response to passive movement of limbs. Short trains of microstimulation appear to hyperpolarize GPI neurons and increase burstiness and in a subset of border cells this leads to pacemaker-like oscillations. These results suggest the GPI may be a tremorgenic site in the basal ganglia-thalamocortical loop.

**Disclosures:**  
Poster

488. Network Oscillations in Parkinson's Disease: Human Studies

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 488.13/D16

Topic: C.03. Parkinson's Disease

Support: VA |01RX000181
NINDS 5R01NS064040

Title: Apathy and depression networks in Parkinson's disease

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Abstract: Psychiatric comorbidities such as depression, anxiety and apathy are common non-motor symptoms in PD, however they can be difficult to identify because of symptomatic overlap with the associated movement disorder. There is growing evidence that abnormal connectivity in the default mode network is linked both to PD and depression. We hypothesized that measures of depression and apathy would correlate with different DMN related networks that were disrupted in PD. We measured depression (Geriatric Depression Score) and apathy (Frontal Lobe Personality Score) in 18 right handed, right side dominant PD patients and 17 age-matched controls. For resting state MRI we used a 3T Siemens TIM Trio MR system to collect T1-weighted MP-RAGE and echo planar functional T2*-BOLD images. Image preprocessing and analysis was performed in SPM 8, and first-level functional connectivity analysis was performed with the CONN toolbox. Second-level analyses correlating cognitive test scores and connectivity of medial prefrontal and dorsal anterior cingulate cortex seeds were performed in AFNI using 3dRegAna. The PD group (mean=4.9, SD=4.3) reported significantly more depression than controls (mean=1.9, SD=1.5; F(1, 33)=7.5, p<0.01). There were no differences in apathy scores between the PD (mean=25.6, SD=6.9) and control groups (mean=23.1, SD=6.3). For the MPFC seed in the PD group, depression scores were negatively correlated with connectivity between MPFC and striatum as well as right insula. In the PD group, apathy scores were negatively correlated with connectivity between MPFC and anterior cingulate as well as left insula. For the dACC seed in the PD group, bilateral insula was negatively correlated with...
dACC connectivity and GDS. We also observed a positive correlation between dACC connectivity and GDS in the posterior cingulate. Bilateral insula, anterior cingulate and medial frontal gyrus were also negatively correlated with dACC connectivity and apathy score. In general, connectivity decreased as depression and apathy increased. Networks overlapped (cingulate and insula), while striatal involvement was unique to depression and medial frontal gyrus connectivity was unique to apathy. Our results represent a potential biomarker for depression in PD that could improve the assessment of disease progression and treatment efficacy, as well as the identification of disease subgroups.


Poster

488. Network Oscillations in Parkinson's Disease: Human Studies

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

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Topic: C.03. Parkinson’s Disease

Support: VA I01RX000181

NINDS 5R01NS064040

Title: Cognitive and motor switching networks are disrupted in Parkinson’s disease

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Abstract: Set switching deficits are a well-described consequence of Parkinson’s Disease (PD). Here we sought to determine the degree to which activity in networks related to motor and cognitive task switching differs in PD compared to healthy controls. In an event-related fMRI experiment, 18 right handed, medicated, early stage, right side dominant PD patients and 19 age-matched controls performed a motor and cognitive switching task. Trials consisted of a colored (yellow or pink) shape (square or circle) presented beneath a word cue. Written cues indicated the stimulus attribute to be identified (“COLOR” or “SHAPE”; adapted from Shook et al., 2005). Participants used fingers on their right hand to press the left button on a button box to indicate “square” or “yellow” and the right button for “circle” or “pink”. On the subsequent trial, a new
randomly generated colored shape would appear. Thus, a given trial could involve no switch, a motor switch (different key press), and/or a cognitive switch (respond to different attribute) relative to the previous trial. Brain images were acquired on a 3T Siemens Trio MRI scanner using a gradient-echo echoplanar imaging pulse sequence and high-resolution, T1-weighted anatomical MP-RAGE sequence. Statistical analysis was performed using SPM8 and AFNI. Comparing fMRI activity during motor switch vs. non-switch trials in controls revealed activity in superior and middle frontal gyri (SFG/MFG), right inferior frontal gyrus (rIFG) and the caudate nucleus, consistent with previous findings. In contrast, the PD group exhibited reduced activity in the left insula, with greater relative activity in the rIFG and left premotor cortex compared to controls. Trials involving a cognitive switch were related to increased activity in SFG/MFG as well as the left insula, putamen, anterior cingulate (ACC), precuneus, and premotor cortex compared to non-switch trials in controls. In PD compared to controls, activity was decreased in the left insula as in the motor switch condition, and also in the ACC and caudate nucleus. Thus, motor and cognitive switching appeared to engage overlapping networks involving frontal (SFG/MFG and premotor cortex) and striatal regions. Motor switching additionally involved the IFG, whereas the insula, ACC and precuneus were selectively active for cognitive switching. The PD group showed relative underactivation in the insula for both types of switching, with additional underactivation in the ACC and caudate for cognitive switching. These patterns of underactivation in PD likely reflect functional deficits in cognitive control networks involved in task switching and response selection.


Poster

488. Network Oscillations in Parkinson's Disease: Human Studies

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 488.15/D18

Topic: C.03. Parkinson’s Disease

Support: VA I01RX000181

NINDS 5R01NS064040-02

Title: Processing speed deficits have far reaching consequences in PD

Authors: *B. HALL, H. NGUYEN, C. HIGGENSON, K. SIGVARDT, L. ZHANG, N. MALHADO-CHANG, E. DISBROW;
Abstract: Processing speed is a dopamine dependent measure of cognitive proficiency that has been shown to increase with age. Parkinson’s disease (PD) is a dopaminergic neurodegenerative disorder that is associated with aging, possibly putting people with PD at higher risk for processing speed deficiency. Thus, we hypothesize that processing speed is increased in those with PD without dementia compared to controls. We studied a group of 68 participants with PD (35 males, 33 females) and 50 controls (26 males, 24 females) between the ages of 55 and 75. Groups were matched for age, years of education, and premorbid IQ. Subjects with PD were medicated and in relatively early stages of the disease. Subjects underwent a battery of neuropsychological tests, including measures of processing speed, attention, memory, cognitive flexibility, activities of daily living, and quality of life, as well as motor performance. Processing speed was evaluated using the Symbol Digit Modalities Test. Between groups comparisons were made using Multivariate Analysis of Variance (MANOVA), and age, daytime sleepiness and motor performance (functional Dexterity Test) were used as covariates in the analysis as possible confounding factors. Processing speed was significantly decreased in the PD group (Mean(SD) 44.3 (11.9) items completed in 90 sec.) compared to controls (Mean(SD) 52.4 (8.2) items completed in 90 sec.; F(4, 113)=12.78, p<0.0001). Additionally, maximum likelihood factor analysis revealed two factors in PD: cognitive and motor. The items that loaded heavily on the cognitive pattern were measures of trail-making, switching, inhibition, and processing speed. Those that loaded heavily on the motor pattern were manual dexterity, mobility, and processing speed. Interestingly, increased processing speed was also associated with increased difficulty with activities of daily living (UPDRS II; R= -.426, p=0.001) and instrumental activities of daily living (R= -.653, p<0.0001) as well as decreased self report quality of life (R= -.279, p=0.024). Our results indicate that processing speed is negatively affected in PD. This deficit has far reaching consequences, contributing to motor and cognitive deficits associated with the disease as well as every-day activities and quality of life.


Poster

488. Network Oscillations in Parkinson’s Disease: Human Studies

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 488.16/D19
Title: Differences in circle drawing rate in PD persons that show impairment in repetitive finger movement

Authors: *A. F. ZAMAN, E. STEGEMOLLER; Kinesiology, Iowa State Univ., Ames, IA

Abstract: Repetitive finger movement is a clinical tool used to assess severity, progression, and treatment efficacy of Parkinson’s disease. Many persons with Parkinson’s disease (PD) demonstrate movement impairment in repetitive finger movement at rates greater than 2 Hz which is not improved with dopaminergic medication. Impaired finger movement can significantly impact the performance of daily living activities such as writing. The purpose of this study was to examine circle drawing at rates both above and below 2 Hz (120 beats per minute) in persons with PD with and without repetitive finger movement impairment. Sixteen persons diagnosed with mild to moderate PD completed a series of circle drawing tasks. Participants used their dominant hand to draw circles 1) at two different sizes (1, and 2cm), and 2) at three different speeds (self-selected pace, 70 beats per minute, and 140 beats per minute). The circle drawing tasks were presented in a random order. Participants were seated in a comfortable position and were allowed to change the angle of the paper to match their preferred writing position and wrote using a pen with an electromagnetic sensor attached to the tip. Based upon performance on the repetitive movement task (differences in movement rate and movement amplitude), participants were divided into groups of with and without impairment. The total area (width x height) of each circle and rate of circle drawing was calculated and averaged across conditions and participants. A two-tailed paired t-test (α = 0.05) was used to compare the means. The results showed that there were significant differences between groups for circle drawing rate, but not for circle area. Future analysis examining the change in area is needed. These results suggest that those persons with PD that demonstrate greater impairment in repetitive finger movement performance may also show greater impairments in handwriting.

Disclosures: A.F. Zaman: None. E. Stegemoller: None.

Poster

488. Network Oscillations in Parkinson's Disease: Human Studies

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 488.17/D20

Topic: C.03. Parkinson’s Disease
Support: Hartman Parkinson’s Research Foundation Pilot Grant

Title: Aberrant neural activity during cognitive control in association with Parkinson’s disease

Authors: *P. MANZA*¹, G. SCHWARTZ², S. ZHANG², C.-S. R. LI², H.-C. LEUNG¹; ¹Stony Brook Univ., Stony Brook, NY; ²Yale Univ., New Haven, CT

Abstract: Parkinson’s disease (PD) is a neurodegenerative disorder characterized by hallmark motor problems but also cognitive deficits. Individuals with PD experience problems with cognitive control of behavior, resulting in a detriment to their quality of life. Cognitive control is the ability to flexibly regulate behavior in the face of distraction, temptation, or surprise, in order to achieve behavioral goals. However, cognitive control deficits have been difficult to treat because the underlying neural mechanisms are unclear. To address this issue, we utilized functional magnetic resonance imaging while individuals performed the Stop-Signal Task. A visual stimulus (Go) is presented on every trial, to which subjects would make a speedy button press. However, a stop signal is presented on a minority (25%) of trials soon after the go signal at a variable delay, which requires withholding of the go response. Twelve individuals in earlier stages of PD (age 63 +/- 9 years; 9 Male, UPDRS part III motor scores 23.7 +/- 9.3, Hoehn & Yahr stage 1.9 +/- 0.3, all drug naïve or “off” PD medications for 12 hours pre-study) and 12 age/sex-matched control subjects participated in the study. We found that stop-signal reaction time, the main measure of stopping ability, was significantly longer in the PD group than in the control group. This cognitive control deficit is not due to general motor slowing in the PD group as no significant differences were found in overall reaction time. Compared to controls, individuals with PD also showed reduced activation on stop trials in several prefrontal and posterior parietal areas and subcortical regions. Finally, we found that neural responses in dorsomedial frontal cortex during post-error go trials were significantly associated with stop-signal reaction time in the control group, but not in the PD group. These findings suggest that individuals with PD show reduced neural activity during stopping and impaired neural responses during error adaptation, which normally guide goal-directed behaviors in healthy adults.


Poster

488. Network Oscillations in Parkinson’s Disease: Human Studies

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 488.18/D21

Topic: C.03. Parkinson’s Disease
Support: NIH Grant R15NS08744701A1

Title: Characterization of essential tremor throughout the upper limb

Authors: *A. PIGG*, D. W. GEIGER*, S. K. CHARLES;*1,2*
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Abstract: Although Essential Tremor (ET), which primarily affects the upper limbs, is one of the most prevalent movement disorders, we do not currently know where in the upper limb the tremor tends to originate (mechanically), how it propagates throughout the upper limb, and where it manifests most severely. The long-term goal of this study is to characterize the distribution of ET patients' tremor from their shoulder to their wrist, simulate the propagation of their tremor across these degrees of freedom (DOF), and estimate the mechanical origin of their tremor (i.e. which DOF or muscle). Here we present a method for characterizing tremor throughout the upper limb and present a preliminary characterization in a small number of patients with mild ET. Ten subjects with mild ET participated in this study. Each subject was instrumented with electromagnetic motion capture sensors on their hand, distal forearm, distal upper arm, and scapula. Subjects were asked to place their upper limb in 16 different postures and hold each posture for 15 seconds. The set of 16 postures were repeated 4 times. We used inverse kinematics with compensation for soft-tissue artifacts to estimate the joint angles in each DOF. Joint angle data were analyzed in the frequency domain to calculate a number of tremor measures, including the power between 4 and 12 Hz (Narrow-band Area, NBA) and over the entire spectrum above 2 Hz (Wide-band Area, WBA), as well as any significant peaks. Mixed-model ANOVA tests were performed to investigate the effects of DOF, posture, repetition, gravitational torque, and subject characteristics on tremor measures. NBA and WBA varied significantly between DOF, being greatest in the elbow and forearm, intermediate in the shoulder, and lowest in the wrist. NBA and WBA also varied significantly with posture, although no pattern was discernable. Only 5% of observations had significant peaks, with 49% of peaks occurring in wrist flexion-extension and 39% occurring in wrist radial-ulnar deviation. Peak frequency was quite stereotyped (5.7 Hz ± 1.3Hz). Repetition had no significant effect, indicating that tremor measures were consistent over the duration of the experiment. Effects of gravity and demographic factors on measures were mixed and did not present discernible patterns. This preliminary characterization suggests that tremor may be focused in a subset of upper limb DOF, being greatest (in terms of power) in elbow flexion-extension and forearm pronation-supination, and most concentrated (with peaks at a stereotyped frequency) in wrist flexion-extension and radial-ulnar deviation. We are currently characterizing more ET patients and simulating tremor propagation.

A one-year phase I trial to evaluate the safety and feasibility of implanting autologous peripheral nerve grafts into the substantia nigra in subjects with Parkinson’s disease undergoing Deep Brain Stimulation surgery and treatment

Authors: C. G. VAN HORNE1,2,3; J. E. QUINTERO1,3; J. T. SLEVIN1,4,5; J. A. GURWELL4; *G. A. GERHARDT6,1,2;

Abstract: In Parkinson’s disease (PD), the substantia nigra undergoes a loss of dopaminergic cells and cell function that, in part, manifests into the outward symptoms of PD. We have an ongoing Phase I trial with the primary endpoint to examine the safety and feasibility of implanting an autologous peripheral nerve graft into the substantia nigra of PD patients undergoing deep brain stimulation (DBS) surgery. Schwann cells from the peripheral nervous system may serve as potential sources of neurotrophic factors including GDNF, NGF, BDNF, and NT-3, and peripheral nerve grafts to the CNS may provide an opportunity to directly deliver neurotrophic factors in areas affected by neurodegenerative diseases. Multi-stage, DBS surgery targeting the subthalamic nucleus was performed using standard procedures. After the DBS leads were implanted, a section of sural nerve (approximately 5mm in length) containing Schwann cells was excised and unilaterally delivered, using a custom-designed cannula, into the area of the substantia nigra. Adverse events were continuously monitored. Quality of life and Unified Parkinson’s Disease Rating Scale (UPDRS) evaluations were monitored preoperatively and at 1, 3, 6, 9, and 12 months after surgery. We have implanted 8 of 8 participants (average age: 62.9 ± 9.2 years; duration with the disease: 9.8 ± 9.2 years; Mean ± SD) with no significant adverse events. Immediate, postoperative magnetic resonance scans did not indicate evidence of abnormal tissue disruption. For the six participants who have completed the 12 month study,
UPDRS Part III (motor) scores off medication/off stimulation were 23.4 ± 12.4 points while at baseline they were 33.1 ± 7.6 points (moderate clinically important differences are defined as >5 points, Shulman et al. 2010). On medication/on stimulation scores were 13.3 ± 9.4 points at baseline and 9.0 ± 6.2 at 12 months. All the while, medication levels decreased from 863 ± 593 daily levodopa equivalents, preoperatively, to 37.5 ± 91.9 after 12 months. Based on our initial safety outcomes and early efficacy results, combining Schwann cell delivery with DBS therapy may provide a means of offering neuroregenerative therapy that augments the benefits of DBS in patients with PD.

Disclosures: C.G. van Horne: Other; Medtronic. J.E. Quintero: Other; Medtronic. J.T. Slevin: None. J.A. Gurwell: None. G.A. Gerhardt: 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.; Medtronic.

Poster

488. Network Oscillations in Parkinson's Disease: Human Studies

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 488.20/D23

Topic: C.03. Parkinson’s Disease

Support: Gifts to the Brain Restoration Center

Tom Dupree for Parkinson’s Disease Research

National Center for Advancing Translational Sciences, through grant UL1TR000117

Title: Using deep brain stimulation surgery as an avenue for providing neuroregenerative therapy to alter the progression of Parkinson’s disease

Authors: J. R. LAMM1, J. E. QUINTERO2,3, A. J. ANDERSON4, J. T. SLEVIN2,4,5, G. A. GERHARDT1,2,3, *C. G. VAN HORNE1,2,3;


Abstract: For over 10 years, deep brain stimulation (DBS) therapy has been used to ameliorate the symptoms of Parkinson’s disease (PD). However, many PD-related symptoms, especially non-motor symptoms, are not relieved through the use of DBS. In addition, DBS therapy has not been shown to halt or reverse the progression of the disease; therefore, new therapies need to be
developed to enhance the therapeutic benefits of DBS. One challenge in that development is finding a means to deliver biologic therapy (such as cell transplants or gene therapy). Based on our current results in a Phase I trial involving delivery of peripheral nerve tissue to the substantia nigra in conjunction with DBS, we think delivery of biological therapy at the time of DBS surgery will prove to be safe and feasible. Our approach was to deliver Schwann cells, a potential, alternative source of neurotrophic factors from the peripheral nervous system. We found that the wealth of results of previous non-clinical, pre-clinical, and early clinical trials showing that neurotrophic factors help restore dopaminergic cell function indicate that neurotrophic factors could serve the role of a therapy that complements DBS. After injury, Schwann cells release a host of growth factors including GDNF, NGF, BDNF, and NT-3. The added benefit of using Schwann cells is that patients can supply their own tissue thereby minimizing the risk for immune rejection. One of the possibilities of this type of surgery and therapy is that other brain areas, ones affected in patients with PD, could be targeted for receiving peripheral nerve grafts to potentially deliver neurotrophic factors and alter non-motor PD-related symptoms. An example of this is the basal forebrain where a loss of cholinergic cell function in PD is associated with cognitive impairment, gait disturbance, and increased incidence of falls. Based on an initial Phase I trial, DBS surgery may provide an avenue for the delivery of biologic therapy that augments the benefits of DBS in patients with PD.

**Disclosures:** J.R. Lamm: None. J.E. Quintero: Other; Medtronic. A.J. Anderson: None. J.T. Slevin: None. G.A. Gerhardt: None. C.G. van Horne: Other; Medtronic.

**Poster**

488. Network Oscillations in Parkinson's Disease: Human Studies

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

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**Topic:** C.03. Parkinson’s Disease

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John A. Blume Foundation

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NSF EEC-1028725
Two modalities of adaptive deep brain stimulation in Parkinson’s disease patients implemented using the Activa® PC+S investigational neurostimulator and Nexus-D System Interface

Authors: *A. VELISAR¹, J. A. HERRON³, Z. BLUMENFELD², E. J. QUINN², M. H. TRAGER², H. J. CHIZECK³, H. BRONTE-STEWART²;
¹Neurol., Stanford Univ., Stanford, CA; ²Neurol., Stanford Univ., Palo Alto, CA; ³Dept. of Electrical Engin., Univ. of Washington, Seattle, WA

Abstract: Objective: Different control variables may be needed for adaptive deep brain stimulation (aDBS) for different phenotypes in Parkinson’s disease (PD). We report the performance of aDBS driven by 1) a neural variable (local field potential (LFP) power) (naDBS) and 2) a kinematic variable (synchronized contralateral hand angular velocity (ang vel) using an inertial measurement unit (IMU) from a LG® Watch) (kaDBS). Methods: Two PD patients (akinetic rigid (AR) and tremor dominant (TD)) were implanted with the Activa® PC+S (Medtronic, Inc.; IDE and IRB approved) neurostimulator. The Nexus-D System Interface communicated with a computer implementing stimulation. Patients were off medication and OFF DBS at baseline. LFP rest beta power (AR) or tremor (TD) was determined during a 60s rest period. naDBS (AR patient): Blocks of “stimulation ON” (2 min) were separated by periods of “stimulation OFF” (15s). The last 5s of a “stimulation OFF” period was used to sense neural activity and measure LFP power in low beta (12-20Hz). If LFP power > 55% of baseline, the next stimulation block voltage (Vstim) was increased; if < 22% of baseline, Vstim was decreased. Wrist flexion-extension (WFE) tasks were performed before, at mid-point, and at the end of naDBS. kaDBS (TD patient): Two kaDBS trials with different Vstim ramp rates (fast and slow) were performed. Hand tremor was monitored in real-time using the IMU. The hand ang vel magnitude in 3-7Hz band was measured at a 5Hz rate and compared to a threshold. If hand tremor > threshold, the Vstim was incremented towards 2V, and if under, Vstim was decreased to 0V. Results: naDBS: The sensed beta power was always > 55% baseline so all stimulation blocks had the max voltage (3V). The WFE ang vel was 254 deg/s before naDBS, 1928 deg/s at mid-point, and 2422 deg/s at the end. kaDBS: Less Vstim was delivered during fast ramp (FR) kaDBS (63%) and slow ramp (SR) kaDBS (67%) versus continuous DBS at 2V, which would amount to one year of extra battery life. During FR kaDBS, the Vstim range was 0-2V with many high-low oscillations; during SR kaDBS, Vstim rose slowly with fewer oscillations and tended to settle around 0.8V (range 0-1.4V). Tremor was present 52% time during SR kaDBS and 56% during FR kaDBS but was stronger during SR kaDBS than FR kaDBS (One way RM ANOVA, P=0.001) and reduced during each kaDBS vs baseline (both P=.001). Conclusions: These experiments demonstrate feasible applications of aDBS using an implanted sensing neurostimulator in PD patients with both neural and kinematic control variables. These results provide preliminary evidence of the potential efficacy of adaptive neuromodulation in clinical practice for different PD phenotypes.

Poster

488. Network Oscillations in Parkinson's Disease: Human Studies

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Topic: C.03. Parkinson’s Disease

Support: John A. Blume Foundation

Medtronic, Inc. provided the investigational devices only

Title: Subthalamic nucleus beta oscillations are attenuated for up to one hour OFF neurostimulation after six months of chronic deep brain stimulation

Authors: *M. H. TRAGER1, E. J. QUINN1, Z. BLUMENFELD1, A. VELISAR1, M. MILLER KOOP1, L. SHREVE1, C. KILBANE3, J. M. HENDERSON2, C. H. HALPERN2, H. BRONTE-STEWART1;
1Dept. of Neurol. and Neurolog. Sci., 2Dept. of Neurosurg., Stanford Univ., Stanford, CA; 3Dept. of Neurol., Case Western Reserve Univ., Cleveland, OH

Abstract: Background: Subthalamic nucleus (STN) local field potential (LFP) recordings demonstrate oscillatory neuronal activity and synchrony in the beta (13-30 Hz) band in the resting state in Parkinson’s disease (PD). Beta synchrony may be attenuated for up to 40 seconds after 5 minute (min) periods of high frequency STN deep brain stimulation (DBS), but it is unknown whether and for how long attenuation persists after turning OFF chronic DBS. We examined whether resting state STN beta band synchrony in PD patients is attenuated after turning OFF chronic DBS and whether this persists for 60 min. Methods: Ten PD subjects underwent bilateral implantation of STN DBS leads connected to a sensing neurostimulator (Activa® PC+S, lead model 3389, Medtronic, Inc. FDA IDE-, IRB-approved). Subjects were tested in the resting state off medication/OFF DBS immediately prior to initial activation of DBS (baseline) and after 6 months (m) of continuous DBS. Five subjects were also tested after 12 m of continuous DBS. STN LFPs were recorded at baseline and immediately after DBS was turned off (0 min) and every 15 min thereafter for at least 60 min. A one-sample t-test (normal data) or one-sample signed rank test (non-normal data) was used to compare power at baseline to the 0 and 60 min recordings, and a Wilcoxon signed rank test was used to compare power between the 0 and 60 min recordings at 6 m. The Unified Parkinson’s Disease Rating Scale
(UPDRS III) was performed pre-operatively (off medication) and at the 6 m visit after DBS was OFF for at least 60 min. Results: Synchronous recordings of kinematic and neural data were used to select periods of at least 10 seconds of data without movement; 4 sides were excluded (2 had intermittent tremor and 2 sides were not stimulated). Baseline beta band power was significantly attenuated immediately (0 min) and 60 min after DBS was turned off at 6 m (P<0.05 for both). There was no significant difference between beta band power at 0 min compared to 60 min after DBS was turned off. UPDRS scores were 42.4 ± 8.9 pre-operatively and 39.9 ± 15.2 after DBS was turned OFF for 185 ± 36.1 min. The interval between UPDRS assessments was 405.5 ± 75.2 days. Five subjects had data after 12 m of DBS: 4 of the 6 sides had lower beta power at 0 min than at baseline, and 3 had less beta power 60 min after DBS was turned off than at baseline. Conclusions: Resting state beta synchrony was attenuated immediately and 60 min after chronic DBS was turned OFF 6 m after initial activation of DBS. There was no increase in pre-operative UPDRS scores even after DBS had been OFF for > 3 hrs. Persistent attenuation of beta band power after chronic DBS suggests that chronic DBS exerts long term effects on neural synchrony in the STN.

Disclosures: M.H. Trager: None. E.J. Quinn: None. Z. Blumenfeld: None. A. Velisar: None. M. Miller Koop: None. L. Shreve: None. C. Kilbane: None. J.M. Henderson: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Intelect Medical, Nevro Corp. F. Consulting Fees (e.g., advisory boards); Intelect Medical, Nevro Corp. C.H. Halpern: None. H. Bronte-Stewart: None.

Poster

488. Network Oscillations in Parkinson's Disease: Human Studies

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 488.23/D26

Topic: C.03. Parkinson's Disease

Support: John A. Blume Foundation

Title: Characterization of resting state beta to high frequency oscillation phase amplitude coupling within the human subthalamic nucleus in Parkinson’s disease

Authors: *M. MALEKMOHAMMADI1, L. SHREVE1, Z. BLUMENFELD1, A. VELISAR1, B. C. HILL1, J. M. HENDERSON2, C. H. HALPERN2, H. BRONTE-STEWARD1;
1Neurol. and Neurolog. sciences, 2Neurosurg., Stanford Univ., Stanford, CA
Abstract: Objective: Phase amplitude coupling (PAC) of beta (13-30 Hz) and high frequency oscillations (HFO: 200-400 Hz) in the subthalamic nucleus (STN) may be involved in Parkinson’s disease (PD) pathophysiology. Investigation of PAC is necessary before it can be called a biomarker for PD. We explored beta-HFO PAC in the resting state in a large well-characterized dataset of PD subjects. Methods: Intra-operative STN local field potentials (LFP) were recorded after placement of deep brain stimulation (DBS) leads (Medtronic Inc, model 3389) in 30 PD subjects (50 STNs) (4 kHz sampling frequency, 400 Hz Low pass filtered). Rest periods without artifact, tremor, or voluntary movement, were selected. Power spectral density analysis was done using multi-taper method (1 second windows, 90% overlap, 1 Hz frequency resolution) and PAC was calculated by modulation index (MI) (phase: 5-35 Hz with 2 Hz bandwidth; amplitude: 50-400 Hz with 15 Hz bandwidth). 100 surrogate data sets were generated to assess the statistical significance of MI values at P < 0.05. Phase of coupling was calculated and its distribution was tested for non-uniformity (Rayleigh test, P < 0.05). The time variability of PAC was studied in 5 seconds non-overlapping time windows. Results: 42 STNs (84%) showed significant beta-HFO PAC (P < 0.05). The Rayleigh test showed deviation of phase distribution from uniform circular distribution (P < 0.05), confirming the presence of a preferred phase for the coupling. The more clinically affected side (MA) (defined by lateral UPDRS III/OFF stimulation and off medication) exhibited stronger PAC in 14 subjects (out of the 20 with bilateral recordings). PAC was spatially specific across the DBS lead and both its magnitude and preferred phase were variable over time across subjects. Conclusions: Resting state beta-HFO PAC exists in the STN in PD and is stronger on the MA side. Moreover PAC is dynamic over time exhibiting temporal correlation with beta power. These results provide additional support for potential pathophysiological role of aberrant PAC in PD and its application for adaptive DBS.
Disclosures:  M. Malekmohammadi: None. L. Shreve: None. Z. Blumenfeld: None. A. Velisar: None. B.C. Hill: None. J.M. Henderson: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Intelect Medical, Nevro Corp.. F. Consulting Fees (e.g., advisory boards); Intelect Medical, Nevro Corp.. C.H. Halpern: None. H. Bronte-Stewart: None.

Poster

489. Huntington’s Disease Mechanisms I

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 489.01/D27

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: National Science Foundation Graduate Research Fellowship Award

Graduate Program in Neuroscience at The Johns Hopkins University

Thomas Shortman Training Fund Graduate Scholarship

NIH

Title: Aberrant nucleocytoplasmic transport in Huntington’s disease

Authors: *I. AHMED, J. C. GRIMA, C. J. DONNELLY, R. SATTLER, S. H. SNYDER, J. D. ROTHSTEIN;

Johns Hopkins Univ. Sch. of Med., Baltimore, MD

Abstract: Huntington’s disease (HD) is a hereditary and incurable neurodegenerative disorder caused by an expanded CAG repeat in the first exon of the huntingtin (htt) gene, resulting in progressive degeneration of striatal medium spiny neurons. Disease onset and severity are dependent on CAG repeat length with a longer expansion resulting in earlier onset and greater severity. However, the underlying mechanisms by which mutant htt causes the disease have not been fully elucidated. Nonetheless, some studies suggest that nucleocytoplasmic trafficking dysfunction could be a pathogenic contributor. The transport of transcription factors between the cytoplasm and the nucleus is a critical aspect of signal transduction and is especially arduous for neurons due to their highly polarized structure. Molecules smaller than 40 kDa are able to transit passively through the Nuclear Pore Complex (NPC), the only transport conduit between the nucleus and cytoplasm. Larger molecules are actively transported by nuclear transport receptors that recognize nuclear localization and export sequences. During nuclear import, cargo release occurs when the transport receptor interacts with RAN-GTP. During nuclear export, RAN-GTP...
is required to form the transport receptor and cargo complex and cargo is released into the cytoplasm upon GTP hydrolysis of RAN-GTP. This irreversible event is induced by RANBP1 or RANBP2 and RANGAP, which are located at the cytoplasmic filaments of the NPC. As a result, a RAN-GTP gradient between the nucleus and cytoplasm is required for this process. Recent studies have shown that HD exhibits increased levels of the polyglutamine-expanded protein in the nucleus, potentially due to a reduction in nuclear export. More specifically, its been shown that expansion of the N-terminal htt fragment reduces its interaction with TPR, a component of the NPC involved with export. Also, live-cell imaging studies have shown that mutant htt demonstrates reduced dynamics and rates of nucleocytoplasmic transport. Finally, cell culture and transgenic animal models display distortions in nuclear envelope and increases in the clustering of NPCs. Our early studies suggest that mutant htt may disrupt nucleocytoplasmic transport. To this end, we assessed components of this pathway in HD immortalized striatal cell lines and a transgenic animal model of HD. Our preliminary data indicate that selected proteins involved in nucleocytoplasmic trafficking are affected in these disease models as evidenced by nuclear aggregation that co-localizes with mutant htt as well as a disruption in the RAN-GTP gradient. This study suggests a defect in nucleocytoplasmic transport in HD.


Poster

489. Huntington's Disease Mechanisms I

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 489.02/D28

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: The University of Texas Medical School at Houston

Hereditary Disease Foundation

Title: Cytoplasmic sphingosine-1-phosphate pathway modulates neuronal autophagy

Authors: *J. F. MORUNO MANCHON¹, E. E. FURR-STIMMING², S. FINKBEINER³, A. S. TSVETKOV⁴, ⁵
¹Neurobio. and Anat., ²Neurol., Univ. of Texas Med. Sch., Houston, TX; ³Gladstone Inst. of Neurolog. Dis. and the Taube/Koret Ctr. for Neurodegenerative Dis. Res., San Francisco, CA; ⁴Neurol. and Physiol., Univ. of California, San Francisco, CA; ⁵The Univ. of Texas Grad. Sch. of Biomed. Sci., Houston, TX
Abstract: Autophagy is an important degradative process that eliminates long-lived proteins, protein aggregates and damaged organelles. Autophagy is required for maintenance of neuronal homeostasis, and its dysregulation is involved in many neurodegenerative disorders. Autophagy is therefore a promising target for blunting neurodegeneration. We searched for novel autophagic pathways in primary neurons and identified the cytosolic sphingosine-1-phosphate (S1P) pathway as a regulator of neuronal autophagy. S1P is a cytoprotective lipid messenger generated by sphingosine kinase 1 (SK1) in the cytoplasm. We found that SK1 expression enhances the flux through autophagy and the S1P-metabolizing enzymes decrease the flux. When autophagy is stimulated, SK1 relocalizes to endosomes/autophagosomes in neurons. Expression of a dominant-negative form of SK1 inhibits autophagosome formation. In a neuron model of Huntington’s disease, pharmacologically elevating the levels of S1P decreased the accumulation of a substrate of autophagy mutant huntingtin and protected neurons from mutant huntingtin-induced neurotoxicity. These results identify the S1P pathway as a novel regulator of neuronal autophagy and provide a new target for developing therapies for neurodegenerative disorders.


Poster

489. Huntington's Disease Mechanisms I

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 489.03/D29

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Huntington Society of Canada

Title: Mutant Huntingtin mediated repression of antioxidant gene expression is associated with sequestration of Nrf2 in aggresomes

Authors: *R. C. CUMMING, L. TINDALE, C. LI;
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). We previously demonstrated that overexpression of mutant Huntingtin (mHtt) in PC12 cells leads to elevated ROS production and a concomitant decrease in transcription of the gene encoding the antioxidant protein peroxiredoxin 1 (Prx1). Interestingly, treatment with the FDA-approved compound dimercaptopropanol (DMP) prevented mHtt-mediated inhibition of
antioxidant gene expression and neurotoxicity. Nrf2 is a transcription factor responsible for regulating expression of a diverse array of antioxidant genes including Prx1. Nrf2 is normally maintained at very low levels by its negative regulator Keap1, which facilitates the sequential ubiquitination and degradation of Nrf2 by the proteasome. Electrophiles and oxidants can disrupt the Keap1-Nrf2 interaction, resulting in the stabilization and nuclear translocation of Nrf2. Following induction of mHtt expression in PC12 cells, we observed a sequestration of both Nrf2 and Keap1 in mHtt containing aggresomes in the cytosol. However, DMP treatment attenuated the sequestration of Nrf2, but not Keap1, in aggresomes and also promoted increased nuclear localization of Nrf2. DMP treatment strongly increased expression of Nrf2 transcriptional targets. These observations suggest that mHtt either directly or indirectly promotes recruitment of Nrf2 to cytoplasmic aggresomes thereby preventing activation of the antioxidant response; an event countered by DMP exposure. 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 a previously unknown intracellular target of DMP and indicates that this FDA approved compound may have relevance for the treatment of HD and other neurodegenerative disorders.

Disclosures: R.C. Cumming: None. L. Tindale: None. C. Li: None.

Poster

489. Huntington's Disease Mechanisms I

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 489.04/D30

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH Grant NS041669

 NIH Grant AG019206

 NIH Grant NS045016

Title: Studying turnover of mutant huntingtin in neuronal and glial cells at subcellular level

Authors: *T. ZHAO¹, Y. HONG³, S. LI², X.-J. LI³;
¹Human Genet., Emory Univ., Atlanta, GA; ²Emory Univ., atlanta, GA; ³emory Univ., Atlanta, GA

Abstract: Huntington’s disease (HD) is an autosomal dominant, neurodegenerative disease that affects one in every 10,000 Americans. About 200,000 Americans are at risk of inheriting the
disease from affected parents. HD patients are characterized by motor, cognitive and neuropsychiatric abnormalities. HD is caused by the expansion of the trinucleotide CAG (>37 units) encoding an expanded stretch of polyglutamine (PolyQ) in the N terminal region of mutant huntingtin (mhtt). Mhtt is neurotoxic and induces neuronal death by disturbing gene expression, axonal transport, and mitochondrial function. Mhtt is prone to forming insoluble aggregates. Appearance of mhtt aggregates is indicative of the accumulation of mhtt. In HD, progressive emergence of mhtt aggregates in neurons is observed. Compared to neurons, fewer and smaller mhtt aggregates are found in astrocytes. Furthermore, the mhtt aggregates preferentially form in neuronal neurites and nuclei, and few aggregates form in the cytosol of soma. This implicates that degradation rates of mhtt in different subcellular compartments are uneven. In order to study degradation rates of mhtt in subcellular compartments, we conjugate dendr2, a photoconvertible fluorescent protein, to the N-terminal fragmented mhtt (Htt230-130Q) and wild-type htt (Htt230-23Q) that is used as the control. Dendra2 is irreversibly photoconverted from a green to a red fluorescent state with 405nm light in the neuronal compartments. After photoconversion, decline of red signal over time is used to measure the degradation rates of Htt230(130/23Q)-dendra2 in the subcellular compartments. In the present study, we used brain slice and primary culture models and found that mhtt is degraded faster than wild-type htt in the cytosol of soma in neurons. However, expanded polyQ stabilizes mhtt in neurites. By contrast, mhtt is cleared faster than wild-type htt in both cytosol of soma and processes of astrocytes. Our data demonstrates differential clearance rates of mhtt in distinct subcellular compartments, and indicates that astrocytes and neurons cope with mhtt in different ways.

Disclosures: T. Zhao: None. Y. Hong: None. S. li: None. X. Li: None.

Poster

489. Huntington's Disease Mechanisms I

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 489.05/D31

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: Role of O-linked beta N acetylglucosamine modification in Huntington’s disease

Authors: *K. MAROSI, R. WU, M. P. MATTSON;
NIH, Baltimore, MD

Abstract: Huntington Disease (HD) manifests with impaired motor and cognitive functions and psychiatric symptoms. HD is caused by a CAG repeat expansion in the huntingtin (Htt) gene that codes for polyglutamine in the huntingtin protein. The mutant huntingtin protein causes
abnormalities in cellular proteostasis and impairs cellular metabolism resulting in neuronal death. O-GlcNAcylation is a posttranslational modification by the O-linked N-acetylglucosamine moiety at proteins. O-GlcNAc transferase (OGT) catalyzes the addition of the sugar moiety to the protein and O-GlcNAcase (OGA) catalyzes the sugar removal. O-GlcNAc acts as a nutrient sensor that couples metabolic status to cellular regulation of signal transduction, transcription, and protein degradation. Aberrant O-GlcNAcylation has been implicated in many diseases including neurodegenerative disorders. Here we performed studies aimed at advancing an understanding of the role of O-GlcNAc modification in the HD pathology. We used immortalized striatal precursor cells expressing normal Htt (Q7) or mutant Htt (Q111) as a model of HD. We found that Q111 cells exhibit slower proliferation rate compared to Q7 cells. Q111 cells showed altered metabolism including reduction in glycolytic capacity and mitochondrial respiration. The Q111 cells showed enhanced capase 3 activation, ROS production and increased vulnerability to serum-deprivation induced stress compared to Q7 cells. This phenotype was associated with elevated p53 levels, which may play an important role in HD pathology. We found that suppressing O-GlcNAc signalling by inhibiting OGT enzyme activity with BADGP rescues the stress-mediated cell death and improves glycolitic function in Q111 cells. In addition, enhancing the global O-GlcNAc signaling with glucosamine treatment resulted in the accelerated death of Q111 cells. We showed that p53 is modified by O-GlcNAc which can potentially regulate its activity and stability. Currently we are determining whether suppression of O-GlcNAc modification on p53 with either pharmacological (BADGP) or genetic tools can potentially enable its ubiquitination and subsequent degradation by the proteasome, which might lead to the prevention of p53-mediated cell death in HD. Since O-GlcNAc modification can fluctuate greatly in response to varying nutrient availability, it is important to understand how dietary interventions might be implemented to suppress pathogenic events involving O-GlcNAcylation in HD.


Poster

489. Huntington's Disease Mechanisms I

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 489.06/D32

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: National Natural Science Foundation of China (No. 81371417)
Title: Lycium barbarum polysaccharide attenuates the cell toxicity of mutant huntingtin through activation of AKT pathway

Authors: *F. FANG;
Dept. of Human Anat. and Embryology, Huazhong Univ. of Sci. & Technol., Wuhan, China

Abstract: Huntington’s disease (HD) is an inherited neurodegenerative disease caused by abnormal expansion of CAG repeats in the first exon of IT15 gene encoding huntingtin (Htt). Clinically, HD is characterized by motor dysfunction, psychiatric disturbance and cognitive deterioration to dementia. Reduction of AKT phosphorylation or inhibition of AKT activity has been found to be involved in cell death induced by mutant Htt. Lycium barbarum polysaccharide (LBP), the main bioactive component of Lycium barbarum, has been reported to play neuroprotective roles through enhancing phosphorylation of AKT in neural injuries including neurodegenerative diseases. Here we report that, treating with LBP can significantly improve motor behavior of HD transgenetic mice, B6C3-Tg(HD82Gln)81Dbo/J Tg mice, and increase cell viability of HEK293 cell stably expressing mutant Htt 160Q (HEK293-160Q cell). Nissl staining shows that there are more Nissl's bodies in the neurons in the cortex and hippocampus of Tg mice treated with LBP than in those of Tg mice without treatment. Furthermore, we found that treatment with LBP increases the phosphorylation of Thr 308 and Ser 473 in AKT and Ser 9 in GSK3β in the cortex, hippocampus and striatum of the TG mice. The upregulation of phosphorylation of Ser 2448 in mTOR is also detected in the hippocampus and striatum. In HEK293-160Q cells, the phosphorylation of Thr 308 and Ser 473 in AKT, Ser 9 in GSK3β and Ser 2448 in mTOR is also significantly increased. Our findings suggest that LBP could alleviate the cell toxicity of mutant huntingtin through activation of AKT pathway, indicating the potential of LBP in treating HD. Keywords: Huntington's disease, AKT, Lycium barbarum polysaccharide

Support: This work was supported by the National Natural Science Foundation of China (No. 81371417).

Disclosures:  F. Fang: Other; National Natural Science Foundation of China (No. 81371417).

Poster

489. Huntington's Disease Mechanisms I

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 489.07/D33

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: CIHR MOP-137041
Title: Regulation of endoplasmic reticulum stress and ribosome biogenesis in a yeast model of Huntington’s disease

Authors: *Y. JIANG, P. LAJOIE; Anat. and Cell Biol., The Univ. of Western Ontario, London, ON, Canada

Abstract: Huntington’s disease (HD) is a neurodegenerative disorder caused by the expansion of polyglutamine (PolyQ) repeats in the huntingtin protein. The expansion results in increased mutant huntingtin protein aggregation, leading to various cellular dysfunctions and ultimately to neuronal cell death. Previous data have identified accumulation of misfolded proteins in the endoplasmic reticulum (termed ER stress) as a major contributor to polyQ toxicity in both mammalian and yeast models of HD. Upon ER stress, down regulation of ribosome biogenesis is a highly conserved coping mechanism by which cells can reduce the amount of nascent proteins to maintain homeostasis. However, the impact of toxic polyQ proteins on ribosome biogenesis and protein translation is unclear. In the well characterized yeast model of HD, this may involve the activation of cell wall integrity pathway (CWI), a signaling cascade homologous to the MAP kinase pathways in human cells. Interestingly, upon polyQ expression, yeast cells expressing an HD-associated polyQ length (72Q) have an obvious growth defect but growth arrest was not associated with cell death. We also showed that cells with expanded polyQ are more sensitive to ER, heat shock and cell wall stresses. Furthermore, we observed a re-localization of Sfp1, a regulator of ribosome biogenesis, from nucleus to cytoplasm in cells expressing expanded polyQ. Therefore we postulate that polyQ expression in yeast impairs translation and cell growth by repressing ribosome biogenesis through CWI activation. Understanding the mechanism of polyQ-induced cell growth arrest will lay a foundation for further research on the mechanisms involved in polyQ toxicity in mammalian systems.

Disclosures: Y. Jiang: None. P. Lajoie: None.

Poster

489. Huntington's Disease Mechanisms I

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 489.08/D34

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIA/NIH AG031153

NIA/NIH AG019206
Title: Molecular mechanism underlying defective BDNF secretion from astrocytes expressing mutant huntingtin

Authors: *Y. HONG*¹, T. ZHAO², X.-J. LI³, S. LI²;
¹Human Genet., ²Emory Univ., Atlanta, GA; ³Emory Univ., Atlanta, GA

Abstract: Huntington’s disease (HD) is a fatal, inherited, neurodegenerative disorder that affects one in every 10,000 Americans. However, there is no effective treatment for HD to date, in part because the pathogenic mechanism driving the disease is incompletely understood. A mutant form of the huntingtin protein (htt), in which a polyQ repeat region is greatly expanded, is a critical molecular feature of the disease. The huntingtin protein is necessary for multiple cellular functions, including gene transcription and vesicle trafficking. However, mutant htt is toxic to neurons. Most studies of neurodegenerative diseases have been focused on neurons because degeneration is observed mainly in neuronal cells. However, the survival of neuronal cells is also supported by glial cells such as astrocytes. One important role of astrocytes is to synthesize and release brain-derived neurotrophic factor (BDNF), which is vital for neuronal survival, development and function. Mutant htt is found in astrocytes both in the brains of HD patients and mouse models of the disease; however, little is known about the pathogenic role of mutant htt in astrocytes. We have found that mutant htt inhibits BDNF secretion from astrocytes. However, how mutant htt impairs BDNF secretion from astrocytes and contributes to the preferential loss of striatal neurons in HD remains unknown. In this study, we used a transgenic HD mouse model (GFAP-160Q) that specifically expresses N-terminal mhtt in astrocytes to study the effect of mutant htt on BDNF secretion. Western blot and real-time PCR results show no significant difference of BDNF level in astrocytes between WT and GFAP-160Q mice. However, ELISA results demonstrate that the secretion level of mature BDNF is decreased in the culture medium of astrocytes from GFAP-160Q mice compared with WT. These results indicate that mutant htt may impair secretion of BDNF from astrocytes, which might contribute to the neuronal dysfunction and degeneration in HD. The mechanism of decreased BDNF secretion is under investigation.

Disclosures: Y. Hong: None. T. Zhao: None. X. Li: None. S. Li: None.

Poster

489. Huntington's Disease Mechanisms I

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 489.09/D35

Topic: C.04. Neurodegenerative Disorders and Movement Disorders
Title: Calcium dependent regulation of Drp1 expression in Huntington’s disease

Authors: *J. JEON, H. SEO;
Hanyang Univ., Ansan, Gyeongggi, Korea, Republic of

Abstract: Huntington’s disease (HD) is a neurodegenerative disorder with progressive degeneration of GABAergic neurons in the striatum. HD is characterized by involuntary movements, chorea, dystonia, changes in personality and cognitive decline. HD is caused by CAG repeat expansion in the huntingtin gene. Recent studies suggest that abnormal mitochondrial dynamics are involved in HD pathogenesis. In this study, we detected how resveratrol, an antioxidant, regulate mitochondrial dynamics in HD model mice. We observed improved mitochondrial function by ATP elevation and attenuation of cellular oxidative stress levels. Especially, Drp1, a mitochondrial fission protein, showed increased expression level in resveratrol administered YAC128 HD mice. We also detected increase in mitochondrial length by resveratrol. Our results demonstrated that the intracellular calcium level affected mitochondrial dynamics by regulating Drp1 expression. Furthermore, down regulation of Drp1 altered mitochondrial morphology. These results suggest that calcium dependent regulation of Drp1 expression not only improves mitochondria function, but aides in the recovery of HD through alteration of mitochondrial morphology.

Disclosures: J. Jeon: None. H. Seo: None.
Title: Cdk5-mediated mitochondrial fission: A key player in dopaminergic toxicity in Huntington’s disease

Authors: M. CHERUBINI, M. PUIGDELLIVOL, J. ALBERCH, *S. GINES-PADROS;
Med. School, Univ. of Barcelona, Barcelona, Spain

Abstract: The molecular mechanisms underlying striatal vulnerability in Huntington’s disease (HD) are still unknown. However, growing evidence suggest that mitochondrial dysfunction could play a major role. In searching for a potential link between striatal neurodegeneration and mitochondrial defects we focused on cyclin-dependent kinase 5 (Cdk5). Here, we demonstrate that increased mitochondrial fission in mutant huntingtin striatal cells can be a consequence of Cdk5-mediated alterations in Drp1 subcellular distribution and activity since pharmacological or genetic inhibition of Cdk5 normalizes Drp1 function ameliorating mitochondrial fragmentation. Interestingly, mitochondrial defects in mutant huntingtin striatal cells can be increased by D1 receptor activation a process also mediated by Cdk5 as down-regulation of Cdk5 activity abrogates the increase in mitochondrial fission, the translocation of Drp1 to the mitochondria and the raise of Drp1 activity induced by dopaminergic stimulation. In sum, we have demonstrated a new role for Cdk5 in HD pathology by mediating dopaminergic neurotoxicity through modulation of Drp1-induced mitochondrial fragmentation, which underscores the relevance for pharmacologic interference of Cdk5 signaling to prevent or ameliorate striatal neurodegeneration in HD.

Disclosures: M. Cherubini: None. M. Puigdellivol: None. J. Alberch: None. S. Gines-Padros: None.

Poster

489. Huntington's Disease Mechanisms I

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 489.11/D37

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: Neuroprotective effects of MicroNeurotrophins in Huntington's disease cellular models

Authors: *K. A. MUELLER1, K. E. GLAJCH1, V. PRABHAKAR1, A. GRAVANIS2, G. SADRI-VAKILI1;
Abstract: Neurotrophic factors (NTFs) are a group of molecules that are important for the development, maintenance, and survival of neurons. NTFs exert their beneficial effects on neurons by binding to tyrosine kinase (Trk) receptors and activating pro-survival cascades. In Huntington’s disease (HD), NTF signaling is dysfunctional and may underlie neuronal death in both animal models of HD and in people living with the disease. Therefore, Trk ligands have been proposed as therapeutic agents for the treatment of HD. Importantly, previous studies in cellular and animal models of HD have demonstrated that NTF treatment is neuroprotective. However, the key challenge in the field of NTF therapy is drug delivery to the central nervous system (CNS) since NTFs are large, charged proteins that do not cross the blood-brain barrier (BBB). Despite the practical challenges of administration, the use of NTFs or NTF analogues remains a promising potential therapeutic given the significant potency of NTFs in preventing cell death and stimulating cell function in animal models of neurodegenerative disease. Currently we are investigating the effects of a novel class of compounds known as MicroNeurotrophins (MNTs) in cellular models of HD. These compounds are small agonists of NTF receptors that can penetrate the BBB. Thus MNTs have immense potential for the treatment of neurodegenerative disorders such as HD. Our results demonstrate that MNT treatment decreases caspase 3 activation, increases mRNA expression of downregulated genes, and enhances autophagy in mutant STHdh{111/111} cells compared to wild-type STHdh{7/7} cells. Furthermore, MNT treatment increases AKT levels as well as huntingtin phosphorylation. Together these findings demonstrate that MNTs confer neuroprotection by decreasing caspase activation, improving transcription and increasing autophagy in cellular models of HD.


Poster

489. Huntington’s Disease Mechanisms I

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 489.12/D38

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: CIHR MOP 102517

Title: Uncoupling GluN2B-NMDA receptors from PSD-95 by Tat-NR2B9c peptide in Huntington’s disease corticostriatal co-culture
**Authors:** *C. BUREN, L. ZHANG, L. RAYMOND;* Psychiatry, The Univ. of British Columbia, Vancouver, BC, Canada

**Abstract:** Huntington’s disease (HD) is a progressive neurodegenerative disorder, which results from an expansion in the CAG repeat region of the Huntingtin (Htt) gene. Multiple studies suggest that mutant Htt (mHtt) expression leads to a deficiency in the major astroglial glutamate transporter and an elevation in extrasynaptic NMDA receptor (NMDAR) expression, especially those containing GluN2B subunit, in striatum. These changes may contribute to NMDAR overactivation and cell death. Uncoupling of PSD-95 from GluN2B with a disrupting peptide, NR2B9c, can rescue the increased vulnerability of HD striatal neurons to NMDA-induced apoptosis, suggesting the peptide has therapeutic potential in HD. However, little is known about the effects of this peptide on synaptic function. Here, we investigated whether TatNR2B9c treatment to weaken the PSD-95/GluN2B interaction can ameliorate synaptic signaling changes in striatal neurons in corticostriatal co-culture from the YAC128 HD mouse, a model that expresses human Htt of 128 repeats. Surprisingly, the peptide reduced rather than improved pro-survival signaling, as reflected in nuclear levels of phospho-cAMP Responsive Element Binding protein (pCREB). Moreover, 1-hour treatment with TatNR2B9c decreased synaptic GluN2B-NMDAR; effects on synaptic GluN2A-NMDAR are being assessed. Currently, we are using whole-cell patch clamp recording to test whether NR2B9c peptide reduces synaptic NMDAR function, which may contribute to impaired survival signaling. In addition, we will assess whether this peptide rescues other changes associated with mHtt expression in striatal neurons, such as enhanced extrasynaptic NMDAR. Together, these studies will lay the foundation for a preclinical trial of Tat-NR2B9c peptide in HD mice.

**Disclosures:** C. Buren: None. L. Zhang: None. L. Raymond: None.

**Poster**

489. Huntington's Disease Mechanisms I

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 489.13/D39

**Topic:** C.04. Neurodegenerative Disorders and Movement Disorders

**Support:** ISTP/Canada/CNPq

FAPEMIG

PRPq/UFMG
Title: Characterization of the role of N-type calcium channels in Huntington’s disease

Authors: *L. B. VIEIRA*, F. R. SILVA, R. P. M. SANTOS, E. M. L. BATISTA, F. M. RIBEIRO

Abstract: Huntington’s disease (HD) is an autosomal dominant neurodegenerative disorder caused by a polyglutamine expansion in the amino-terminal region of the huntingtin protein (Htt). HD patients exhibit neurodegeneration on the caudate-putamen and neocortical regions of the brain, as well as symptoms including motor alterations, cognitive decline, psychiatric disturbances and inevitable death. Although neurodegeneration is pointed as the main cause of HD symptoms, cognitive decline can be observed even before detectable levels of neuronal cell loss. Several studies indicate that mutated Htt (mHtt) can promote Ca2+ signaling disturbances, which are closely related to the death of striatal neurons. It has been demonstrated that wild type Htt can interact with and also modulate N-type calcium channels (Cav2.2), which play an important role in pre-synaptic neurotransmitter release. Our main question is how N-type calcium channels are affected by full-length mHtt expression and whether it could account for altered neurotransmission in HD. Using synaptosomes from HD mouse model, BACHD, we observed an increase on glutamate release in the striatum. Applying to these synaptosomes, a potent and selective Cav2.2 blocker (w-CgTx GVIA), we noticed that the increase in glutamate release is abrogated by this N-type calcium blocker. Through immunoblotting and biotinylation, we analyzed the total expression as well as the membrane expression of Cav2.2 in the cortex and striatum of BACHD mice. We observed that Cav2.2 total expression is decreased in older mice, but increased in the striatum membrane in younger mice. Co-immunoprecipitation was applied for measuring the interaction between Cav2.2 and mHtt, or Gβγ and Syntaxin 1A, that both have a binding site on this channel and also contribute to its regulation. Our results showed that Cav2.2 interacts less with mHtt in the striatum. On the other hand, Cav2.2 interacts more with Syntaxin 1A and less with Gβγ in BACHD mice. Taking together our results, we may suggest that the neuronal death that occurs in HD has a possible involvement of N-type calcium channel with mHtt, Gβγ and Syntaxin 1A proteins during the early phase of HD.

Program#Poster#: 489.14/D40

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: Exploring the role of wild-type HTT in human oligodendrocytes by functional and microarray analyses

Authors: *Y. TAY*\textsuperscript{1}, S. NAMA\textsuperscript{2}, P. SAMPATH\textsuperscript{2,3}, M. POULADI\textsuperscript{1,4};  
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Abstract: White matter (WM) atrophy in Huntington disease (HD), an autosomal dominant neurodegenerative disorder, is well documented, with WM changes occurring many years before the onset of clinical manifestations. Despite these observations, the significance of WM changes towards the pathogenesis of HD has not been fully explored. To better understand the role of wild-type huntingtin (HTT) in WM biology and function, we performed microarray gene expression profiling of the immortalised human MO3.13 oligodendroglial cells with lentiviral-mediated shRNA knockdowns of wild-type HTT. Knockdown of wild-type HTT by more than 80% of its endogenous levels resulted in the differential expression of 1289 genes (734 up-regulated and 555 down-regulated) with a fold change of at least 1.5 and a false discovery rate corrected p-value of <0.05 in three independent biological samples (using two different HTT shRNA constructs). Gene Ontology (GO) term analysis revealed dysregulation in processes integral to normal oligodendroglia function such as chemotaxis, cell adhesion, metabolic processes and cell morphogenesis. A number of oligodendrocyte related genes were also dysregulated following knockdown of wild-type HTT. We verified the differential expression of a subset of genes involved in these processes using quantitative real time PCR analysis and also assessed how cell proliferation, survival and chemotaxis may be impaired following HTT knockdown. In all, this is the first study that specifically explores the potential roles of wild-type HTT in the context of oligodendrocytes.

Disclosures: Y. Tay: None. S. Nama: None. P. Sampath: None. M. Pouladi: None.

Poster

489. Huntington’s Disease Mechanisms I

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 489.15/D41

Topic: C.04. Neurodegenerative Disorders and Movement Disorders
Title: Effect of total or allele-specific silencing of normal and mutant HTT in derivatives of Huntington’s disease human pluripotent stem cells

Authors: *M. CHERIF*1, M. JARRIGE2, S. GRIBAUDO2, A. MARTEYN3, A. PLANCHERON3, S. AUBERT3, M. REY4,5, C. MONVILLE3, N. DÉGLON4,5, A. PERRIER2;
1Inserm/ UEVE UMR861, ISTEM, Evry, France; 2Inserm UMR861, ISTEM, Evry, France; 3CECS, ISTEM, Evry, France; 4Clin. neurosciences (DNC), 5Neurosci. Res. Ctr. (CRN), Lausanne Univ. Hosp. (CHUV), Lausanne, Switzerland

Abstract: Huntington’s disease (HD) is an autosomal dominantly inherited disorder characterized by late onset psychiatric, cognitive and motor deficits. The main pathological hallmark of HD is the massive neuronal loss of GABAergic projection neurons (medium spiny neurons) of the striatum. As the disease progresses marked neuronal loss is as well observed in the cortex (in particular cortico-striatal and cortico-thalamic pyramidal neurons housed in the cortical layer V and layer VI, respectively) and other subcortical structures. HD is caused by a CAG repeat expansion in the exon 1 of the HTT gene from a normal range of usually 16-20 to more than 35. This gives rise to an expanded polyglutamine tract in HTT protein. The mutant protein (mut-HTT) is prone to aggregation and is associated with a gain of toxic function and a dominant negative activity on normal HTT function. As HTT is a scaffold protein involved in many pathways, mutation within HTT has an impact on several cell mechanisms. The signaling pathways linking the genetic cause of HD and mut-HTT to the cascades of cellular processes leading to the striatal and cortical neurons dysfunction and death are not fully understood. Here we explored the specific role of HTT and the impact of HD mutation in various derivatives of human pluripotent stem cells (hPSC). We used hPSC-derivatives featuring phenotype equivalent to that of cells in brain regions most affected by the disease such as telencephalic neuroepithelial progenitor cells, cortical and striatal neurons as well as control cells such as retinal pigmented epithelium cells (RPE) a highly polarized epithelial type of cells (not affected in HD patients). We designed DOX-inducible shRNA lentiviral vectors targeting single nucleotide polymorphisms present in the human HTT gene to selectively target the mut-HTT isoform (previously described in Drouet et al 2014) and DOX-inducible shRNA vectors targeting total HTT. We next used these inducible shHTT-viruses on WT and HD-hPSCs and on their derivatives to explore the specific impact of HTT loss of function and mut-HTT toxic or dominant negative effect on different cellular functions. Initial whole genome transcriptomic analysis using pan-allele or allele-specific silencing vectors confirmed a role of HTT in many life-supporting cellular processes such as neural or retinal functions and cell adhesion. These results comfort the position of HTT as a multifunctional scaffold protein necessary to many life-supporting cellular events and highlight the relevance of an inducible silencing system to better understand the role of HTT and the impact of HD in cell differentiation and maturation.

Title: Mitochondrial Division Inhibitor 1 and mitochondria-targeted molecules MitoQ and ss31 protects against mutant htt-induced mitochondrial toxicities in Huntington’s disease neurons

Authors: *X. L. YIN, M. MANCZAK, Y. SUNEETHA, R. KANDIMALLA, A. PANDEY, C. KURUVA, P. REDDY; GIA, TTUHSC, Lubbock, TX

Abstract: BACKGROUND Huntington’s disease (HD) is a fatal, progressive neurodegenerative disease with an autosomal dominant inheritance, characterized by chorea, involuntary movements of the limbs, and cognitive impairments. Recent studies have found that mutant Htt interacts with mitochondria fission protein Drp1, causing excessive fragmentation of mitochondria, leading to abnormal mitochondrial dynamics and neuronal damage in HD-affected neurons. Some progress has been made in developing molecules that can reduce excessive mitochondrial fragmentation while maintaining the mitochondrial dynamics and mitochondrial function and synaptic activity. The objective of this study was to determine the protective effects of mitochondrial division inhibitor 1 (Mdivi1) and mitochondria-targeted molecules - MitoQ and SS31 in striatal neurons that stably express mutant Htt STHHDhQ111/Q111.

METHODS Using Seahorse XF96 extracellular flux analyzer, we assessed real-time oxygen consumption ratio (OCR) in STHHDhQ111/Q111 neurons that were treated and untreated with Mdivi1, MitoQ and SS31. Further, using biochemical methods, cell viability, apoptosis, mitochondrial function and mitochondrial DNA (mtDNA) and nuclear DNA ratios were assessed in in STHHDhQ111/Q111 neurons that were treated and untreated with Mdivi1, MitoQ and SS31.

RESULTS The XF96 extracellular flux analyzer analysis of spare respiratory capacity, particularly oxygen consumption ratio was significantly increased in STHHDhQ111/Q111 neurons treated with Mdivi1, MitoQ and SS31 relative to untreated STHHDhQ111/Q111 neurons. The Cell viability was significantly increased in STHHDhQ111/Q111 neurons treated with Mdivi1 (P=0.039), MitoQ (P=0.0367) and SS31 (P=0.0438) relative to untreated STHHDhQ111/Q111 neurons. The cell apoptosis assays revealed that both apoptotic and necrotic cell deaths were reduced in
STHDhQ111/Q111 neurons treated with Mdivi 1, MitoQ and SS31 compared to untreated neurons. Mitochondrial ATP production was increased and hydrogen peroxide levels reduced in STHDhQ111/Q111 neurons treated with Mdivi 1, MitoQ and SS31 relative to untreated STHDhQ111/Q111 neurons. mtDNA-nDNA ratios analysis revealed that mtDNA copy numbers were reduced in STHDhQ111/Q111 neurons treated with Mdivi 1, MitoQ and SS31 relative to untreated STHDhQ111/Q111 neurons. CONCLUSIONS These findings suggest that Mdivi1 and MitoQ and SS31 protective against mutant-Htt induced mitochondrial and synaptic damage in HD neurons and that Mdivi1 and MitoQ and SS31 are promising molecules to study using HD mouse models and also to study in clinical trials using HD patients.


Poster
489. Huntington's Disease Mechanisms I

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 489.17/D43

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Huntington Society of Canada

Title: Role of TNFα in regulating corticostriatal synapses in the Huntington’s disease

Authors: *R. DUSEJA1,2, G. M. LEWITUS2, H. F. ALTIMIMI2, M. FRANQUIN2, D. STELLWAGEN2;
1Ctr. for Res. in Neurosci., McGill Univ., Montreal, QC, Canada; 2Dept. of Neurol. and Neurosurg., Ctr. for Res. in Neurosci., Montreal, QC, Canada

Abstract: Huntington’s disease (HD) is caused by mutation of the huntingtin (HTT) gene leading to an expansion of glutamine in the protein. The disease is primarily characterized by the
degeneration of the medium spiny neurons (MSNs) in the striatum. MSNs are broadly classified as direct pathway neurons (primarily expressing dopamine D1 receptors) and indirect pathway neurons (preferentially expressing D2 receptors). Abnormal alterations in the corticostriatal glutamatergic inputs to the MSNs are thought to underlie many striatal dysfunctions, and contribute to the motor deficits that characterize HD. Mechanisms by which mutant HTT alters these synapses are largely unknown. One possibility is that synaptic strength may be regulated by the pro-inflammatory cytokine Tumor Necrosis Factor alpha (TNFα), a well-known component of HD. TNFα levels are elevated in the striatum, cerebrospinal fluid and plasma of the HD patients, but its contribution to the development of the HD is not well characterized. We recently identified TNFα as a novel factor regulating corticostriatal synapses. Here, we investigate the role of TNFα in regulating the corticostriatal synaptic strength in the YAC128 animal model of HD. At pre-symptomatic age (2 months), we observed a decrease level of TNFα protein in the striatum of YAC128 mice. This is accompanied by an increase in synaptic strength (as measured by an increase in AMPA/NMDA ratio) specifically on direct pathway D1-MSNs. No change was observed in the synaptic strength on D2-MSNs nor in release probability, suggesting the change in the synaptic strength is post-synaptically driven. To further investigate the role of TNFα, we generated the YAC mice on TNF null background. Interestingly, our preliminary data indicates that YAC128 mice on TNF null background do not demonstrate an increase in AMPA/NMDA ratio. We are also behaviorally characterizing these mice. So far, our behaviour data suggests that in comparison to WT mice, YAC128 mice have gait alteration. Conversely, YAC128 mice on TNF null background do not demonstrate any alteration in the gait. Overall, our data suggest that TNFα may be regulating synaptic strength and motor output of the striatum during HD.


Poster

489. Huntington's Disease Mechanisms I

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 489.18/D44

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH grant R01 NS040408

Title: Reduced Foxp1 expression as a contributor to Huntington’s disease
Huntington’s disease (HD) is an inherited neurodegenerative disease caused by the abnormal expansion of a CAG repeat in the first exon of the huntingtin gene resulting in a mutant protein with a poly-glutamine expansion. Although mutant huntingtin (mut-Htt) is expressed ubiquitously in the brain, neurodegeneration in HD occurs selectively in the striatum and, to a lesser degree, the cortex. The reason for the selective vulnerability is not known. We propose that reduced expression of the Foxp1 gene is an important contributor to the selective vulnerability of the striatum and cortex in HD. We and others have found that Foxp1 is expressed highly and selectively in the striatum and, to a lesser degree, the cortex. Foxp1 expression is reduced in the striatum and cortex, but not in other brain regions of R6/2 mice. Other labs have reported reduced Foxp1 expression in the striatum of HD patients. Expression of Foxp1 is also reduced in cultured cortical neurons induced to die by oxidative stress, a feature implicated in HD-related neuronal loss, but not in dying cerebellar granule neurons which are not affected in HD. Knockdown of Foxp1 expression induces death in otherwise healthy cortical neurons suggesting that elevated expression of this gene is necessary for neuronal survival. Indeed, restoring elevated levels of Foxp1 protects cortical neurons against mut-Htt neurotoxicity. Foxp1 overexpression also prevents death of cortical neurons resulting from oxidative stress. Besides the commonly studied ~90 kDa isoform, there are two other isoforms that are ~50 and ~70 kDa in size. The ~70 kDa Foxp1 is also expressed highly and selectively in the striatum and cortex, is robustly and selectively downregulated in the striatum and cortex R6/2 mice, and is neuroprotective when overexpressed. Current studies in the lab are focused on investigating the mechanism underlying neuroprotection by Foxp1.

Disclosures: A. Louis Sam Titus: A. Employment/Salary (full or part-time); University of Texas at Dallas. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; NIH grant R01 NS040408.

S. D’Mello: A. Employment/Salary (full or part-time); Southern Methodist University. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; NIH grant R01 NS040408.
Program#/Poster#: 489.19/D45

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH Grant NS064138

CHDI Foundation Early Discovery Initiative

Hereditary Disease Foundation

Title: Nuclear retention of full-length HTT RNA is mediated by splicing factors MBNL1

Authors: *X. SUN1, P. P. LI2, S. ZHU2, R. COHEN2, L. O. MARQUE2, C. A. ROSS2, R. L. MARGOLIS2, D. D. RUDNICKI2;

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2Dept. of Psychiatry, Johns Hopkins Univ. Sch. of Med., Baltimore, MD

Abstract: Huntington’s disease (HD) is caused by a CAG repeat expansion in huntingtin (HTT). Recent evidence suggests that HD is a consequence of multimodal, non-mutually exclusive mechanisms of pathogenesis that involve both HTT protein- and HTT RNA-triggered mechanisms. Here we provide further evidence for the role of expHTT in HD by demonstrating that a fragment of expHTT is neurotoxic in the absence of any translation and that the extent of neurotoxicity is similar to the neurotoxicity of an expHTT protein fragment encoded by a transcript of similar length and with a similar repeat size. In addition, full-length (FL) expHTT is retained in the nucleus. Overexpression of the splicing factor muscleblind-like 1 (MBNL1) increases nuclear retention of expHTT and decreases the expression of expHTT protein in the cytosol. This suggests that MBNL1 plays a role in nuclear export of expHTT RNA. We propose that nuclear RNA export is an important component of the pathogenesis of HD and that preventing the retention of expHTT RNA may have therapeutic potential.


Poster

489. Huntington's Disease Mechanisms I

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 489.20/D46

Topic: C.04. Neurodegenerative Disorders and Movement Disorders
Support: NS066339

Title: Altered lysosomal positioning in a cellular model of Huntington’s disease

Authors: M. L. LU1, *J. WEI2;
1Biomed. Sci., 2Florida Atlantic Univ., Boca Raton, FL

Abstract: Huntington’s disease (HD) is an inherited monogenic neurodegenerative disease caused by an abnormal polyglutamine (polyQ) expansion in the huntingtin protein (Htt). Full length Htt is a 350KDa cytosolic protein that has been demonstrated to regulate the dynamics and trafficking of various subcellular organelles, including mitochondria and lysosomes. In the current study, we investigated the subcellular distribution of lysosomes in two clonal striatal cell lines derived from wild-type (STHdhQ7/Q7, hereafter referred as STHdhQ7) and mHtt (STHdhQ111/Q111, hereafter referred as STHdhQ111) knock-in mice using both anti-lamp1 immunostaining and live-cell imaging with lysoTracker red DND-99. The degrees of lysosome segregation/positioning were quantified. We found that in STHdhQ7 cells lysosomes were distributed throughout the cytosol. In contrast, STHdhQ111 cells display a more segregated lysosome distribution mainly accumulated in the perinuclear regions. The same results were observed in primary fibroblasts derived from a healthy human individual and a HD patient. This perinuclear lysosomal accumulation can be reversed by ectopically expressing normal Htt in HD cells. Further characterizing the functional significance of the increased perinuclear lysosomal accumulation in HD cells, we demonstrate subsequently that basal mTORC1 activity is increased in HD cells. In addition, autophagic influx is also increased in HD cells in response to serum deprivation, which leads to a premature fusion of lysosomes with autophagosomes. Taken together, our data suggest that the increased perinuclear accumulation of lysosomes may play an important role in HD pathogenesis by altering lysosomal-dependent functions.

Disclosures: M.L. Lu: None. J. Wei: None.

Poster

489. Huntington’s Disease Mechanisms I

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 489.21/D47

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: Identification of Cellular Pathways that are Dysregulated in Huntington’s disease
**Authors:** *A. BAHARANI*¹, S. NAPPER²;
¹Dept. of Biochem., ²VIDO, Dept of Biochem., Univ. of Saskatchewan, Saskatoon, SK, Canada

**Abstract:** Huntington's disease (HD) occurs worldwide and the occurrence of the disease is 5 to 7 among 100,000 people. HD is an autosomal dominant, most commonly inherited neurological disorder that includes prominent motor, psychiatric and cognitive symptoms resulting from degeneration of neurons in the striatum and cerebral cortex. The genetic defect is the presence of expanded CAG repeats in the huntingtin gene resulting in long (>36) repeats of the amino acid glutamine in the huntingtin protein. The identification of signal transduction pathways that are dysregulated in HD will be completed through analysis, which will allow us to verify probable kinase targets.

**Disclosures:** A. Baharani: None. S. Napper: None.

**Poster**

**489. Huntington's Disease Mechanisms I**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 489.22/D48

**Topic:** C.04. Neurodegenerative Disorders and Movement Disorders

**Support:** NIMH 1R25 MH101076

**NS066554**

**Title:** PRC2 regulates transcriptional and behavioral phenotypes induced by mutant Huntingtin

**Authors:** *R. J. FENSTER*²,³, A. HEILBUT¹,⁴, R. KULICKE¹,³, A. POWERS¹, L. J. HACHIGIAN¹,³, J. P. MESIROV¹, E. D. KOLACZYK⁴, M. HEIMAN¹;
¹The Broad Inst., Cambridge, MA; ²Dept. of Psychiatry and Human Behavior, Brown Univ., Providence, RI; ³Picower Inst. for Learning and Memory, Cambridge, MA; ⁴Grad. Program in Bioinformatics, Boston Univ., Boston, MA; ⁵Dept. of Brain and Cognitive Sci., MIT, Cambridge, MA

**Abstract:** Huntington’s disease (HD) is the most common inherited neurodegenerative disease and is caused by CAG trinucleotide repeat expansions in the huntingtin gene. Although many neuronal cell types exhibit pathology in HD, medium spiny neurons (MSNs) of the striatum are among the most vulnerable cell populations. The reason for this enhanced vulnerability is unknown but could offer opportunities for therapeutic intervention and a better understanding of the molecular mechanisms underlying mutant Huntingtin action. In both human HD and mouse
models of the disease, MSNs lose expression of cell type-specific markers, including Ppp1r1b (DARPP-32), Drd1a, Drd2, and Penk, suggesting that loss of MSN molecular identity is a hallmark of HD pathogenesis. We have previously shown an increase of polycomb repressive complex 2 (PRC2) activity in mouse models of HD. Here we show preliminary data suggesting that the PRC2-dependent mark H3K27me3 is present at MSN marker genes along with the canonical H3K4me3 mark of transcriptional activation, indicating that these genes are “bivalently” marked. By experimentally increasing H3K27me3 in wild-type mouse striatum, we demonstrate decreased expression of MSN markers and motor phenotypes reminiscent of HD. Our data suggest a model in which the loss of MSN identity and motor phenotypes caused by mutant huntingtin are linked to PRC2 activity.


Poster

489. Huntington’s Disease Mechanisms I

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 489.23/E1

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: The JPB Foundation

5 T32 GM 7484-37

Title: Polyq loss of function in Huntington's disease

Authors: *L. HACHIGIAN*1, A. HEILBUT2, R. FENSTER3, R. KULICKE1, E. KOLACZYK2, J. MESIROV3, M. HEIMAN1,2,3;

1MIT, Cambridge, MA; 2Boston Univ., Boston, MA; 3Broad Inst., Cambridge, MA

Abstract: Huntington’s disease (HD) is a neurodegenerative disorder caused by an expansion of poly-glutamine (or polyQ) repeats in the huntingtin (HTT) gene. Interestingly, striatum and deep layer cortex undergo widespread degeneration while other brain regions are less affected. This differential vulnerability cannot be explained merely by expression of the mutated HTT, as the protein is found across all brain regions. In order to gain insight into the molecular basis of this selective neurodegeneration, we took advantage of the translating ribosome affinity purification (TRAP) methodology to uncover any differences that may underlie this phenomenon. Profiles of 25 different brain regions revealed elevated expression of non-HTT polyQ repeat proteins in
vulnerable HD cell types, namely striatal medium spiny neurons and deep layer cortical pyramidal cells, in contrast to less vulnerable regions. These proteins are mostly nuclear-localized transcription factors and RNA-interacting proteins. It is known that polyQ proteins can interact with expanded HTT, influencing its aggregation and toxicity. Moreover, certain polyQ proteins have been demonstrated to co-aggregate with mutant HTT, leading to a loss of their function which can be rescued by their overexpression. Based on our initial findings, we hypothesized that a cell’s polyQ load might dictate its vulnerability to mutant HTT-induced degeneration. Analysis of HD mouse models reveals co-aggregation of mutant HTT with striatal- and cortical-enriched polyQ proteins identified by TRAP. These proteins lose their typical diffuse nuclear expression in HD, which may interfere with their ability to bind DNA and regulate transcription. Interestingly, this phenomenon of polyQ co-aggregation was specific to mutant HTT as opposed to other expanded CAG repeat proteins. The ataxin proteins contain expanded glutamine repeat stretches in the spinocerebellar ataxia diseases, yet striatal- and cortical-enriched polyQs do not co-aggregate with expanded ataxin proteins in the absence of striatal involvement. Taken together, our work suggests that elevated expression of polyQ proteins defines vulnerable cell populations in HD. The co-aggregation of these proteins with mutant HTT and their subsequent loss of function contributes to the striatal and cortical degeneration observed in HD.

**Disclosures:** L. Hachigian: None. A. Heilbut: None. R. Fenster: None. R. Kulicke: None. E. Kolaczyk: None. J. Mesirov: None. M. Heiman: None.

**Poster**

**490. Autism Spectrum Disorder Models: Novel and Emerging**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program/#Poster#:** 490.01/E2

**Topic:** C.06. Developmental Disorders

**Support:** Simons Foundation (SFARI 314688, A.G.).

The study was funded by the Istituto Italiano di Tecnologia

**Title:** Frontal hypoconnectivity in the 16p11.2 microdeletion autism model

**Authors:** *A. BERTERO*¹, ², G. DAVID², A. LISKA², A. GALBUSERA², M. PASQUALETTI², ³, A. GOZZI²;

¹Univ. of Pisa, Pisa, Italy; ²Cnt. for Neurosci. and Cognitive Systems, Inst. Italiano di Tecnologia, Rovereto, Italy
Abstract: Autism spectrum disorder (ASD) has been often associated to the presence of reduced or aberrant functional brain connectivity as measured with resting state functional Magnetic Resonance Imaging (rsfMRI). However, great heterogeneity exists in the distribution and expression of these alterations, and little is known on the pathophysiological and genetic determinants underlying these deficits. Human chromosome 16p11.2 microdeletion, a trait associated to mild intellectual disability, is the most common gene copy number variation in autism, accounting for approximately 0.5-1% of all ASD cases. By using rsfMRI in a mouse model of human chromosome 16p11.2 microdeletion, we show that this genetic alteration results in circuit specific functional connectivity reductions. Specifically, we show that 16p11.2+/- mice exhibit reduced rsfMRI connectivity between retrosplenial and dorsal prefrontal areas of the mouse “default mode network”. Similarly reduced connectivity was observed between insular and prefrontal portions of the mouse “salience network”. Evidence of reduced thalamo-prelimbic connectivity was also observed. No genotype-dependent inter-hemispheric, fronto-hippocampal, and local connectivity differences were recorded. Collectively, our findings recapitulate hallmark neuroimaging findings in ASD and identify plausible macroscale circuitual alterations underlying some of the cognitive deficits produced by 16p11.2 microdeletion.

Disclosures: A. Bertero: None. G. David: None. A. Liska: None. A. Galbusera: None. M. Pasqualetti: None. A. Gozzi: None.

Poster


Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 490.02/E3

Topic: C.06. Developmental Disorders

Support: NS075062

T32GM008361

IRSF2916

Title: Motor and behavioral phenotypes in a novel transgenic rat model of Rett Syndrome

Authors: *K. PATTERSON, K. ARPS, M. OLSEN; Univ. of Alabama At Birmingham, Birmingham, AL

Abstract: Rett Syndrome is an x-linked neurodevelopmental disorder caused by mutations in the transcriptional regulator MeCP2, affecting approximately 1 in 10,000 girls annually.
Historically, researchers have utilized transgenic mouse models in the study of this disease. Recently, Sage® Labs made available a transgenic rat model of Rett Syndrome (SD-Mecp2tm1sage). This model, which contains a 74 base pair deletion in exon 4 of the MECP2 gene, displays a functional knockout of MeCP2 protein. Here, we track changes in growth as well as motor and behavioral deficits of male and female rats throughout development, and outline the utility of this specific model in the study of Rett Syndrome. Heterozygous females develop symptoms at varying developmental time points, with the majority displaying abnormalities such as increased body weight by 4 months of age. Mutant males are noticeably symptomatic as early as weaning, displaying lethargy and an unkempt appearance. Malocclusion develops in the majority of mutant males by the 4th-5th week of life. Body weights do not differ between mutant and wild type males when animals affected by malocclusion are excluded from analysis. Our data suggest that mutant males demonstrate decreased brain weight by postnatal day 14, and display motor deficits as early as the fourth postnatal week as assessed by Rotor-Rod™ and Catwalk™ analysis. Additional deficits are observed in open field during advanced disease. Batteries of identical tests are underway utilizing females from weaning age through late adulthood.

Disclosures: K. Patterson: None. K. Arps: None. M. Olsen: None.

Poster


Location: Hall A

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Program#/Poster#: 490.03/E4

Topic: C.06. Developmental Disorders

Support: NIH Grant DP5OD009134 (RS)

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Harvard Brain Tissue Resource Center, HHSN-271-2013-00030C

Texas Children’s Hospital (RS)

Autism Speaks (RP)
Loss of MeCP2 in the rat uniquely models regression, impaired sociability, and transcriptional deficits of Rett syndrome

Authors: *R. C. SAMACO¹, S. VEERARAGAVAN⁴, S. M. HAMIILTON³, C. S. WARD⁵, Y.-W. WAN², S. SORIANO¹, M. R. PITCHER⁶, C. M. MCGRRAW⁷, W. YAN¹, J. R. GREEN⁴, L. YUVA⁴, A. J. LIANG¹, J. L. NEUL⁵, D. H. YASUI⁶, J. M. LASALLE⁸, Z. LIU⁹, R. PAYLOR⁴;
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Abstract: Mouse models of the transcriptional modulator Methyl-CpG-Binding Protein 2 (MeCP2) have advanced our understanding of Rett syndrome (RTT). RTT is a 'prototypical' neurodevelopmental disorder with many clinical features overlapping with other IDD/ASD whose pathogenesis may be similar. Therapeutic interventions for RTT may therefore have broader applications. However, the reliance on the laboratory mouse may present challenges in translating findings from the bench to the clinic, and the need to identify outcome measures in well-chosen animal models is critical for preclinical trials. Therefore, we set out to identify disease-relevant neurobehavioral deficits that can be uniquely modeled in a rat model of Rett syndrome, and to compare transcriptional changes in MeCP2 rodent models and human Rett brain tissue. We found that female Mecp2 rats display psychomotor regression of a learned skill and impairments in juvenile play, two behavioral deficits that are unique to the rat model and that are highly relevant to RTT. We also demonstrate that the strategy of analyzing the loss of Mecp2 in both mouse and rat may result in higher predictive validity with respect to transcriptional changes in human RTT brain. These data underscore the similarities and differences caused by the loss of MeCP2 among divergent rodent species which may have important implications for the treatment of individuals with disease-causing MECP2 mutations. Taken together, these findings demonstrate that the Mecp2 rat model is a complementary tool with unique features for the study of RTT and highlight the potential benefit of cross-species analyses in identifying potential disease-relevant preclinical outcome measures.

Title: Neurobehavioral differences and similarities between genetic rodent models of ASD

Authors: *S. VEERARAGAVAN*, J. R. GREEN, S. M. HAMIILTON, L. YUVA, R. C. SAMACO, R. PAYLOR;

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Abstract: Mouse models of autism spectrum disorders (ASD) have been instrumental in our current understanding of the neurobehavioral consequences caused by disease-causing human mutations. Neurobehavioral deficits are prominent in ASD, and the use of genetic tools such as the laboratory mouse, is one approach to identify potential therapies that may improve these impairments. However, given the concerns that findings from mouse models may not necessarily reflect changes that are relevant to the human condition, and the possibility that such models may be sub-optimal for translational studies, we set out to test the neurobehavioral phenotypes in novel rat models of specific ASD genes to determine the extent to which genetic manipulation of these genes in a second mammalian rodent species results in similar and/or different neurobehavioral deficits. Focusing on *Fmr1* male rats, and *Pten, Cntnap2, Nrxn1* and *Met* male and female rats, we evaluated juvenile animals using both conventional neurobehavioral assays and assays that can be uniquely studied in the rat. We found that several of these rat lines displayed phenotypes that are not completely consistent with reported findings in the mouse, including abnormalities in social behavior and rodent communication (ultrasonic vocalizations), two core features of ASD. However, for other secondary features of ASD such as sensorimotor gating deficits and cognitive impairments, we found that some lines displayed alterations that were consistent between rat and mouse ASD models. These data suggest that the ASD rat models may prove to be complementary tools to the existing repertoire of ASD animal models, providing certain unique advantages for studying behavioral phenotypes including social and cognitive assessments. Moreover, given the conflicting data between mouse and rat ASD models, these findings underscore the importance of investigating the consequences of disease-causing human mutations in a variety of animal model systems.
Poster

**490. Autism Spectrum Disorder Models: Novel and Emerging**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 490.05/E6

**Topic:** C.06. Developmental Disorders

**Title:** Assessing the mecp2 (bird) model of rett syndrome across species, sex, and age

**Authors:** D. BRUNNER1, *P. A. KABITZKE1, M. OSBORNE2, A. BARBOZA2, L. THIEDE1, N. ROBERTS1, T. HANANIA1;
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**Abstract:** X-linked MECP2 gene mutations in humans have been shown to be associated with loss of voluntary movements, including speech and hand movements. Male MECP2 homozygous mice are commonly used in preclinical studies and show a distinct phenotype but typically do not survive past 3 months of age and cannot perform many behavioral tests due to their compromised state. The female MECP2 Bird mouse (Mecp2tm1.1Bird) lacks one copy of the Mecp2 excised with Cre-loxP technology, has normal survival and appears quite healthy. However, we found that this model shows increased hindlimb clasping, weaker grip strength, and impaired ability to remain on the rotarod. The female Mecp2 Bird mouse also exhibits decreased acoustic startle response and decreased optokinetic response as measured by turning of the head in the direction of stripe movement, a measure of reflexive behavior and smooth eye tracking. In addition, female Bird mice reproduce the apnea phenotype seen in Rett. Lastly, female Bird Rett mice from 6 weeks of age onward demonstrate a distinct gait phenotype as measured in PsychoGenics’ proprietary NeuroCube® System. This high-throughput system can quantitatively assess a disease phenotype, can quantify drug recovery, and has been validated in many drug discovery and screening studies. A novel model, the Mecp2 heterozygous rat (SAGE) showed similar gait deficits starting at 8 weeks of age, extending the availability of robust models of Rett with face validity.

**Disclosures:** D. Brunner: A. Employment/Salary (full or part-time); PsychoGenics, 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.; IRSF. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual
In humans the 16p11.2 deletion syndrome is caused by a loss of a chromosomal segment that encodes about 25 genes. This microdeletion results in variable deficits and features of autism, developmental delay and intellectual disability are common. Mouse models of 16p11.2 deletion syndrome have been developed and these animals have several behavioral abnormalities and are typically hyperactive. In addition, there is evidence for altered basal ganglia organization in 16p11.2 deletion syndrome mice. Serotonin neurotransmission has long been associated with autism, yet it remains poorly understood if altered serotonin neurotransmission could represent a common deficit in autism generated by distinct genetic or environmental factors. In this study, we evaluated several measures of serotonin neurotransmission in 16p11.2 deletion mice (df+) and their wild-type (WT) littermates (Jackson Labs B6129S-Del(7Slx1b-Sept1)4Aam/J; Horev et al., PNAS 2011). Bred with B6129F1/J for at least 8 generations, df+ mice were present in a Mendelian ratio but weighed less than their WT siblings at postnatal day 28. Behavior in response to an acute stress (swim) was studied, as this stimulus is known to activate the serotonin system. While WT mice habituated during the swim test and showed increasing passive coping with time, df+ mice perseverated with an active coping strategy. HPLC analysis of monoamine content of the dorsal striatum showed that swim
changed the levels of dopamine and its metabolites in both genotypes, but in addition df/+ mice had altered serotonin turnover after the swim. 5-HT2A receptors are involved in basal ganglia function and some drugs used for autism interact with 5-HT2A receptors. To evaluate the role of 5-HT2A receptors in altered behavior we tested the effects of the antagonist, M100907 (Volinanserin). Df/+ mice were more sensitive to M100907 than their WT siblings, and a dose of 0.01 mg/kg attenuated their behavioral abnormalities in the swim. Parallel results were found with locomotor behavior in the open field. Taken together, these findings suggest altered endogenous 5-HT2A receptor tone may exist in df/+ mice, and may contribute to their behavioral phenotype.

Disclosures: C.M. Panzini: None. A.M. Alchahin: None. Y. Guo: None. K.G. Commons: None.

Poster


Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 490.07/E8

Topic: C.06. Developmental Disorders

Support: SNF 155952

Title: Absence of parvalbumin results in an Autism Spectrum Disorder-like phenotype in mice

Authors: *F. FILICE, B. SCHWALLER;
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Abstract: Autism spectrum disorder (ASD) patients are characterized by impairments in social interaction, communication and stereotyped patterns of behavior, interests, and activities. ASD-associated genes often encode proteins that are implicated in synaptic transmission and/or structure. Functionally these changes result in an impairment of neurotransmission at individual synapses and at the network level, in modifications of e.g. the excitation/inhibition (E/I) balance that might be caused by mutations in ASD risk genes during earlier steps in neurodevelopment. Within the global neuronal network, interneurons play a key role in the maintenance of the overall balance of activity and interneuron dysfunctions are linked with cognitive impairment in neuropsychiatric disorders. In particular, the number of fast-spiking interneurons (FSI) expressing the calcium-binding protein parvalbumin (PV) has been reported to be decreased in different well-assessed mouse models of ASD. PV-deficient mice (PV-/- and PV+/-) show ASD-like symptoms similar to ones reported in other “canonical” ASD mouse models (Wöhr et al.,
According to the current view, the decrease in PV-immunoreactive (PV-ir) neurons in mouse ASD models is the result of a “loss” of PV-expressing FSI, leading to a change in the E/I balance. Based on the ASD-like phenotype observed in mice with reduced (PV+/−) or absent PV (PV−/−) expression, we hypothesized that PV-downregulation, i.e. without a loss of the PV neurons, might be sufficient to elicit the ASD-like traits. Stereology-based analyses of a particular extracellular matrix component surrounding most PV-ir neurons, termed the perineuronal nets (PNNs), revealed the number of PNN-positive cells to be unchanged in WT, PV+/− and PV−/−. Thus, a mere down-regulation of PV affecting intracellular Ca2+ signaling appears to be sufficient to result in an ASD-like behavioral phenotype. Current experiments aim to testing whether PV-downregulation and/or PV-neuron loss is the cause for the previously reported decrease in the number of PV-ir neurons in other well-established mouse ASD models. An unchanged number of “PV-FSI” in those genetic models would indicate that PV-downregulation might represent a common/convergent pathway for some types of ASD with different genetic etiologies.

Disclosures: F. Filice: None. B. Schwaller: None.

Poster


Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 490.08/E9

Topic: C.06. Developmental Disorders

Support: Brain/MINDS

NPO Rett Synd Supporting Organization

Title: Generation and analysis of autism model marmoset

Authors: *N. KISHI1,2, K. SATO3, M. OKUNO1,2, H. J. OKANO4, E. SASAKI1,2,3, H. OKANO1,2;
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Abstract: In the human brain, there are two major functional domains. One has been conserved in all mammals through evolution and governs fundamental functions such as reward, emotion and memory; the other is unique to primates, and is acquired through the enlargement of the cerebral cortex governing special functions such as tool use, language, and self-consciousness.
Thus, to properly understand these brain functions, we need appropriate animal models for studying each function. Animal models that are used to analyze brain functions are different in each case. In the former, a reductive approach is adopted based on gene manipulation using models such as genetically-modified fish and rodents, while in the latter, the main approach is psychological and involves complex behavior analysis using non-human primates such as macaque monkeys. Many researchers believed that the complementary nature of genetic engineering technologies in rodent and fish models and cognitive neuroscience techniques in primate research would lead to progress in this research field. However, due to lack of appropriate animal models that can be analyzed in both aspects of the brain’s functions, contact points between these two approaches have been limited. The development of genetically engineered non-human primates has attracted attention for its potential to connect the two research fields. Recently, we succeeded in creating the world’s first transgenic primate using marmosets. This technological breakthrough provides a potential paradigm shift by enabling researchers to analyze both the brain functional domains using various model marmosets. Currently, we are developing a technique for creating knockout marmosets using zinc finger nuclease (ZFN) technology. By combining this technique with the development of cognitive information for marmoset brain analysis, innovative MRI imaging technology and marmoset genetic analysis tools, we created and are analyzing MECP2 mutant marmosets suitable for research on Rett syndrome, to understand the pathogenesis, and to contribute to new therapeutic strategies to treat Rett syndrome.

**Disclosures:** N. Kishi: None. K. Sato: None. M. Okuno: None. H.J. Okano: None. E. Sasaki: None. H. Okano: None.

**Poster**

**490. Autism Spectrum Disorder Models: Novel and Emerging**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 490.09/E10

**Topic:** C.06. Developmental Disorders

**Support:** Nancy Lurie Marks Clinical and Research Fellowship Program in Autism

Robert and Donna Landreth Fund

Lurie Center for Autism

**Title:** Early postnatal treatment with lipopolysaccharide (LPS) as a mouse model of immune-mediated ASD
Authors: A. J. Alexander1, S. M. Landino1, C. J. McDougle2, B. C. Finger1, W. A. Carlezon1;

Abstract: The role of immune responses in the etiology of autism spectrum disorder (ASD) has long been hypothesized. Indeed, it is thought that a “multiple hit” model may apply to ASD whereby multiple immune insults early in life may increase the risk of developing an ASD. To further investigate this hypothesis, we developed a mouse model of immune-mediated ASD using a double-immune challenge approach. Pregnant mice were injected with the viral mimic poly(I:C) (20 mg/kg) on day 12.5 of pregnancy. The offspring were subsequently injected with lipopolysaccharide (LPS) (10 mg/kg) on postnatal day 9.5 to induce a bacterial infection. A battery of tests were performed in a 2x2 factorial design to characterize the behavioral phenotype in relation to the core symptoms of ASD: deficits in communication and social interaction, and increases in stereotyped behaviors. To assess deficits in communication, ultrasonic vocalizations were recorded from male pups during a maternal separation test on postnatal days 10-16 and from adult males at 9 weeks in a female encounter test. In pups, postnatal LPS treatment significantly increased the number of calls emitted, independent of prenatal treatment. However, this altered communication did not persist into adulthood, with no significant difference between groups in a test involving an encounter with a female conspecific. To assess deficits in social interaction, a one-chamber social interaction test was performed using males and females at 8 weeks. In males, postnatal LPS decreased social preference independent of prenatal treatment. There was no effect of prenatal or postnatal treatment on social preference in females. To assess anxiety, both males and females at 10 weeks of age were scored on an open field test. There was a mild anxiogenic phenotype in mice that received postnatal LPS, irrespective of sex or prenatal treatment. Additionally, to assess stereotypic behavior, mice were tested in a marble-burying task at 11 weeks. In males only, postnatal LPS caused an altered pattern of behavior that was independent of prenatal treatment. Our results indicate that postnatal immune challenge with LPS causes alterations in communication, social preference, anxiety and stereotypic behavior. However, prenatal poly(I:C) did not reliably produce any of these ASD-related phenotypes, and did not increase phenotype severity when administered in combination with postnatal LPS, going against the multiple-hit hypothesis. More experiments are necessary to further characterize this model, but these data suggest that immune insults early in postnatal development may produce behavioral changes that can be used to study immune-mediated ASD.


Poster

**Location:** Hall A  

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM  

**Program#/Poster#:** 490.10/E11  

**Topic:** C.06. Developmental Disorders  

**Title:** Effects of cerium oxide nanoparticles on learning and motor behavior in the valproic acid rat model of autism spectrum disorder  

**Authors:** W. E. DECOTEAU, A. E. FOX, J. LICATA, J. PARISE, *A. Y. ESTEVEZ*;  
1Psychology, 2Biol., St. Lawrence Univ., Canton, NY  

**Abstract:** Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by communication deficits, repetitive behaviors, and impairments in social interactions. Increased oxidative stress, possibly from the exposure to environmental toxins has been proposed as a cellular mechanism contributing to the development of ASD. Valproic Acid (VPA) is an environmental toxin that generates oxidative stress and has been linked to ASD. The VPA model is one of the most widely used rodent models of ASD; however, it is unclear whether this model exhibits learning and behavioral deficits similar to those observed in humans with ASD. It is also unknown whether those deficits could be ameliorated by treatment with cerium oxide nanoparticles (CeNPs), a powerful and novel anti-oxidant. In the present experiment, we examined whether *in utero* exposure to VPA (500mg/kg, ip) can generate specific learning and behavioral deficits and, if so, whether pre-treatment with CeNPs (20 mg/kg, iv) can serve as a therapeutic protection against those deficits. Pregnant rats were treated with either CeNPs or vehicle on gestation day 11.5 and then exposed to VPA on gestation day 12.5. A third group was given vehicle injections on both gestation days. Pups from each group were weaned and then tested on a battery of tasks that assessed their motor skill, temporal discrimination, and novelty detection. Abnormal motor behavior, usually in the form of hyperactivity, was observed in the VPA rats across all tasks. Compared to controls, VPA rats displayed poor habituation and re-exploration on the novelty recognition task. Interestingly, VPA rats learned the time discrimination task faster than controls and exhibited transient elevated response rates. Motor skill deficits were moderately ameliorated in animals treated with CeNPs *in utero*. This result is consistent with the therapeutic effect of CeNP that has recently been described in animal models of motor neurodegeneration.  

**Disclosures:** W.E. DeCoteau: C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Cerion. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Cerion NRX. A.E. Fox: None. J. Licata: None. J. Parise: None. A.Y. Estevez: C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Cerion. E. Ownership
Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Cerion NRX.

**Poster**


**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 490.11/E12

**Topic:** C.06. Developmental Disorders

**Support:** Meixner Fellowship Autism Speaks

**Title:** The genetic intersection of neurodevelopmental disorders and shared medical comorbidities-relationships that translate from bench to bedside

**Authors:** A. J. STEVENSON\(^1,2\), *J. PLUMMER\(^1\), P. LEVITT\(^1,2\);
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**Abstract:** The search for the heritability of mental illness and neurodevelopmental disorders (NDDs) has led to the discovery of underlying genetic and cellular mechanisms. While there is brain enrichment in the expression of NDD risk genes, these typically exhibit expression in peripheral tissues as well. Thus, there is potential shared biological liability, which has led to the present hypothesis that there are specific patterns of convergent medical comorbidities related to NDD genes. To explore this possibility, we examined the phenotypes associated with approximately 200 NDD risk genes. We conducted a comprehensive search of Psychiatric Genomics Consortium, ADHDgene, Simons Foundation Autism Research Initiative, Online Mendelian Inheritance in Man, and NCBI Gene databases - PhenGen, ClinVar and the Genetic Testing Registry. The database search included >500 genes, of which 200 were prioritized based on 1) brain expression, 2) citation by multiple studies and/or 3) functional validation. These 200 genes were initially grouped by their primary NDD association, then further stratified by additional genetically-associated organ system disorders and comorbidities. NDD gene network analysis revealed underlying relationships among psychiatric disorders and peripheral comorbidities that include cancer, cardiovascular disease, renal disorders, respiratory disorders and metabolic disorders, demonstrating a broader impact of brain-associated genes in other developing organ systems. Patterns of genes with overlapping comorbidities emerged, highlighting that genetic mutations in patients with NDDs can specifically affect other organ systems. These relationships emphasize the clinical importance of recognizing NDDs as more than simply brain disorders. In an era when personalized medicine is becoming an expected part
of clinical practice, understanding the comorbidities associated with given genetic mutations will facilitate the identification of additional health challenges. Ultimately, data can be used to optimize holistic treatment and management of patients with NDDs.


Poster


Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 490.12/E13

Topic: C.06. Developmental Disorders

Support: EU-AIMS research receives support from the Innovative Medicines Initiative Joint Undertaking (grant 115300), resources composed of financial contribution from the EU FP7 Program, EFPIA companies in kind contribution, and Autism Speaks.

Title: Dissecting autism heterogeneity in developing mice

Authors: *M. KAS;
Univ. Med. Ctr. Utrecht, Utrecht, Netherlands

Abstract: About 1 in 100 children suffers from an autism spectrum disorder (ASD). These neurodevelopmental disorders are clinically defined by impairments in social interaction and by restricted, repetitive and stereotyped behavior. Most of the autistic behaviors become manifest in the first years of life; a time when brain circuits are being shaped by sensory experiences. However, ASD is a highly heterogeneous disorder with respect to severity and variability of symptom expression; some patients show predominantly stereotyped behaviors whereas others may have mainly deficits in social behavior development. As human genetic studies have identified over 200 ASD risk genes, the challenge is to understand how these gene defects lead to individual variation in impaired social interaction and/or stereotyped behavior. By implementing a longitudinal behavioral test battery in mice, we have shown that genetic mouse models for ASD displayed different characteristics of impaired social interaction or stereotyped behavior at different stages in development. Understanding these neurodevelopmental trajectories may open up the possibility of reversing these core phenotypes by means of adequate therapeutic strategies.

Disclosures: M. Kas: None.
Abstract: The Autism Database (AutDB) is a publicly available, manually annotated, modular database that serves as an ongoing collection of genes linked to Autism Spectrum Disorders (ASD). Here, we describe the design, development and integration of the animal model module of AutDB which catalogues ASD-related rodent models. All data is extracted from published, peer-reviewed primary reports. In addition to ASD models, the animal model module also contains rescue models based on existing parent ASD models. The new release of the database also includes rat models in addition to mouse models, since both of these species are widely used in current research. We aim to include other species as the database grows. The metadata is standardized in a phenotypic database (PhenoBase), which is a routinely updated comprehensive list of phenotypic terms (pheno-terms) and experimental paradigms. These pheno-terms reflect the actual research and are divided into categories that align with human ASD phenotypic features. The recently updated PhenoBase has been standardized to about 380 pheno-terms divided into 16 categories. This standardization allows for data mining and bioinformatics analysis that coupled to our large curated dataset can be used to elucidate ASD research trends and etiology.

Title: Is the sympathetic hyperactivity involved in the cardioprotection against myocardial lesions by ischemia and reperfusion in hypertensives animals?

Authors: *F. S. MENEZES RODRIGUES*¹, J. G. P. TAVARES², P. R. ERRANTE², M. C. M. REIS², R. MIRANDA-FERREIRA², L. DE PAULA², B. LUNA FILHO³, A. CARICATI-NETO²;
¹Pharmacol., Univ. Federal De São Paulo - EPM, Diadema, Brazil; ²Pharmacol., ³Cardiol., Univ. Federal de São Paulo - Escola Paulista de Medicina, São Paulo, Brazil

Abstract: INTRODUCTION: It is well known that cardiac lesions by ischemic (I) and reperfusion (R), typical of acute myocardial infarction, compromise cardiac structure and function, and yet that these lesions induce sympathetic hyperactivity (Lameris et al, Circulation, 2000). Although the sympathetic mechanisms could be involved in cardioprotective response (Tavernier et al, Cardiovasc. Res., 2003), the cardioprotector role of these mechanisms remain unclear. OBJECTIVE: Using a study model of arterial hypertension (Spontaneously Hypertensive Rats, SHR), which sympathetic activity is increased (Miranda-Ferreira et al, JPTE, 2009), we decided to investigate if the response to cardiac I/R is altered in situations related to sympathetic hyperactivity such as hypertension. METHODS: Male SHR and its normotensive controls (NWR) of 12 to 16 weeks were anesthetized (urethane 1.25 g/kg, i.p.), kept under mechanical ventilation and submitted to surgical procedures to induce cardiac I/R by means occlusion of left anterior descendent coronary artery (10 min) and reperfusion (120 min). Electrocardiogram (ECG) system was coupled to rats submitted to cardiac I/R to evaluate the incidence of ventricular arrhythmia (VA), atrioventricular blockade (AVB) and lethality (LET). The serum concentration of specific marker of myocardial lesions, creatine kinase-MB (CK-MB), was evaluated. The effects of ischemic preconditioning (preIC) in NWR submitted to cardiac I/R was studied. RESULTS: Compared to NWR, systolic blood pressure and cardiac mass are significantly increased in SHR (51% and 20%, respectively). Incidence of VA, AVB and LET resultant of cardiac I/R was significantly lower in SHR (40%, 20% and 21%, respectively) than in NWR (85%, 79% and 70%, respectively). The increase of serum concentration of CK-MB induced cardiac I/R lesions was lower in SHR (16%) than in NWR (129%). These results suggest that SHR could develop adaptive cardioprotective responses similar those produced by preIC. This hypothesis was confirmed by results obtained in the NWR pretreated with preIC and submitted to cardiac I/R, in which the values of VA (28%), AVB (0%) and LET (14%) were significantly lower compared to NWR submitted to cardiac I/R alone.

CONCLUSIONS: The similar responses to cardiac I/R observed in SHR and NWR pretreated...
with preIC suggest that sympathetic hyperactivity associated to arterial hypertension and cardiac I/R could stimulate a set of adaptive responses able to mitigate myocardial dysfunctions and lesions caused by I/R. Financial support - FAPESP, CAPES and CNPq.

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**Poster**

**490. Autism Spectrum Disorder Models: Novel and Emerging**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 490.15/E16

**Topic:** C.06. Developmental Disorders

**Support:** the Swedish Research Council

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**Title:** Rare inherited genetic variation in a multiplex family with autism and language disorders

**Authors:** *L. JONSSON*¹, C. MINISCALCO², M. JOHNSON², T. MARTINSSON³, J. MELKE¹;

¹Gillberg Neuropsychiatry Centre, Inst. of Neurosci. and Physiology, the Sahlgrenska Acad.,
²Univ. of Gothenburg, Goeteborg, Sweden; ³Dept. of Clin. Genet., Inst. of Biomedicine, Sahlgrenska Univ. Hospital, Univ. of Gothenburg, Gothenburg, Sweden

**Abstract:** BACKGROUND: Genetic studies of autism spectrum disorders (ASDs) support an important role for multiple rare variants in the etiology of these conditions. Identified genetic variants include both copy number variations (CNVs) and single nucleotide mutations. Furthermore, inherited as well as de novo variants seem to contribute to the risk of developing an ASD. METHODS: In our study, we explore both rare single nucleotide mutations and CNVs in a multiplex family with two children with autism. Thirteen family members, of whom several have variable degrees of ASD related conditions and language problems, are included in the analyses. The index children both have an ASD diagnosis as well as language disorder. We used both CNV (Affymetrix 6.0) and whole exome sequencing (Illumina) to identify rare inherited variation. **In vitro** functional assays using COS-1 cells have been performed for one of the identified mutations in the CYP11A1 (p.T369M), coding for the cholesterol side-chain cleavage
enzyme (P450scc). RESULTS: Our main finding is a rare gain-of-function mutation in CYP11A1 (p.T369M) that codes for the first enzyme in steroidogenesis. The mutation was found in both index children and in five other family members. Three rare paternally inherited CNVs have been identified in regions with no or few previously reported CNVs; 6q15 (~40 kb deletion), 11q21 (~75 kb deletion) and 4q34.3 (~57 kb deletion). Only the deletion in the 6q15 region contains a gene; the RNA Guanylyltransferase And 5'-Phosphatase (RNGTT) gene that is involved in transcription regulation.

DISCUSSION: Our exploratory investigation of a family searching for both CNVs and rare single nucleotide mutations using WES has identified both rare CNVs and mutations that may be of importance in this family. In our analyses we did not find any previously identified causal variants for autism, however the identified gain-of-function mutation in CYP11A1 is in line with previous findings suggesting increased steroidogenic activity in autism.


Poster


Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 490.16/E17

Topic: C.06. Developmental Disorders

Support: NIH Grant MH103680

Wadsworth Center

Title: Rescuing forebrain commissure defects in a mouse model of autism: Can we also alter relevant behaviors?

Authors: K. MANLEY1, A. SNYDER-KELLER1,2, G. W. BOTHE1, K. KLUETZMAN1, *V. J. BOLIVAR1,2; 1Wadsworth Ctr., Albany, NY; 2Sch. of Publ. Health, Dept. of Biomed. Sci., State Univ. of New York, Albany, NY

Abstract: Mouse models, such as the BTBR T+ Itpr3tf/J (BTBR) strain, play an important role in the elucidation of the biological mechanisms underlying autism spectrum disorder (ASD) and the development of new therapeutic interventions. BTBR mice display multiple ASD-relevant behavioral abnormalities (e.g., reduced social interactions, impaired communication, increased
repetitive movements). However, the genetic and neurological bases for these behavioral abnormalities remain unknown. As BTBR mice also exhibit corpus callosum (CC) and hippocampal commissure (HC) malformations, we hypothesized that these neuroanatomical phenotypes could be related to ASD-relevant behaviors. We recently developed two CC/HC rescue models on a BTBR genetic background. We developed a congenic mouse line by transferring a locus on the distal end of Chromosome 4 from FVB/NJ (FVB) to BTBR and a C57BL/6 (B6) derived BAC from the same Chromosome 4 locus was used to create a transgenic line. Mice from these two lines were evaluated in a behavioral test battery (open field, zero maze, social approach, self-grooming). We also determined structure size by staining sections using 0.2% gold chloride and measured midsagittal CC and HC area. BTBR mice carrying none, one, or two copies of the FVB locus were evaluated in littermates from the congenic line, and hemizygous B6 BAC transgenic mice were compared to non-transgenic littermates. The congenic mice revealed rescue of the CC and HC phenotypes in the presence of one or two copies of the FVB locus. The B6 BAC transgene was sufficient to rescue CC/HC phenotypes in hemizygous BTBR mice. We are also correlating structure size with performance on each of the behavioral assays. Our research should lead to a better understanding of the biology underlying forebrain commissure development and the subsequent effects on ASD and related behavioral phenotypes.


Poster


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Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

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Topic: C.06. Developmental Disorders

Support: NIH Grant P50 MH078028

Department of Cell and Developmental Biology, Vanderbilt

Title: Changes in cortical wiring in a mouse model of autism

Authors: C. D. M. VARGAS1,2, J. A. MAVITY-HUDSON3, M. J. ROBSON3, J. VEEENSTRA-VANDER WEELE4, M. T. WALLACE5,6,7,2, R. D. BLAKELY6,3,7,2, *V. A. CASAGRANDE5,6,8,7,8.

1Interdisciplinary Program in Neurosci., 2Vanderbilt/NIMH Conte Ctr., 3Pharmacol., 4Pharmacol.,
Abstract: Serotonin (5-HT) is known to play a role in the neural development, affecting cortical connectivity (Lauder, 1990, Bonnin et al., 2007, Bonnin et al., 2011). Due to the long history of 5-HT as a biomarker for autism spectrum disorder (ASD), alterations in 5-HT metabolism have become a focus of interest in attempting to determine the etiology of ASD (Persico et al., 2014). In addition to classical deficits in social communication repetitive behaviors, sensory symptoms are a core diagnostic feature of ASD (DSM-5). Given the importance of 5-HT in early cortical development, and the evidence for sensory disturbances that extend beyond a single sensory system, a plausible hypothesis is that changes in serotonin signaling may play an underappreciated role in the abnormal connectivity patterns in sensory cortices in ASD. To study this question, we used a mouse model with a known Gly56Ala mutation in the serotonin transporter SERT (or 5-HTT), encoded by the SLC6A4 gene. In humans this mutation causes increased serotonin reuptake and has been associated with autism and compulsive behavior. The knock-in (KI) mice were compared to the 129s4/s6 hybrid background mice. The goal was to examine the laminar organization and distribution of thalamocortical axons and serotonin axons in primary visual cortex (V1) and a known multisensory cortical area (V2L). Adult mice from each group were perfused and brains cut in either the coronal or parasagittal plane. Sections were immune-stained for vesicular glutamate transporter 2 (VgluT2) to identify thalamic axons and 5-HT to label serotoninergic axons. Adjacent sections were also stained for cytochrome oxidase (CO) to identify cortical layers. Cortical layers were equally distinct between the KI and WT animals in V1 and V2L in CO sections. In VgluT2 stained sections, however, distinct differences could be seen in V1, with significantly less label in layer 4 and in the superficial layers of the mutants compared to background mice. In V2L VgluT2 was very dense in layer 4 and layer 1 of both groups but did not appear to differ between the mutant and background groups in any cortical layer. In the 5-HT immunostained sections the most obvious difference was in the distribution of axons in V2L. Here, in KI animals the 5-HT labeled axons were much more diffusely distributed within the cortex and showed less clear laminar boundaries. These findings support the idea that alterations in cortical connectivity associated with changes in 5-HT signaling may play an important role in the known pattern of sensory and multisensory deficits in ASD.


Poster

491. Fragile X Syndrome
**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 491.01/E19

**Topic:** C.06. Developmental Disorders

**Title:** Detailed spectral analysis of Fragile X Syndrome mice vocalizations, a model to study speech deficits

**Authors:** *A. BELAGODU, A. JOHNSON, R. GALVEZ;*  
Univ. of Illinois Urbana-Champaign, Urbana, IL

**Abstract:** Fragile X (FX) Syndrome is the leading form of inherited mental retardation. It is caused by the transcriptional silencing of fmr1, the gene which codes for the fragile X mental retardation protein. Patients that have FX have been shown to exhibit numerous behavioral and cognitive impairments, such as ADHD, OCD, and autistic-like behavior (Miller et al., 1999; Cordeiro et al., 2011). In addition to these behavioral abnormalities, FX patients have also been shown to exhibit various deficits in speech and language. Studies in human FX patients have shown that they form abnormal sentence structures and have issues with utterances, increased repetition of sounds and words, and articulation (Largo and Schinzel, 1985; Hanson et al., 1986; Price et al., 2008). To study the biological underpinnings of these speech abnormalities, studies have used a mouse model of the fragile X syndrome. These studies have examined pup ultrasonic vocalizations (USV) due to either maternal or mating separation in FX mice and have shown various differences in the number and duration of vocalizations produced (Rotschafer et al., 2012; Roy et al., 2012; Lai et al., 2014). However, these findings were inconsistent between age groups, and did not provide a detailed investigation into spectral properties of the USVs. Deficits in USV spectral properties, such as mean frequency and frequency modulation rate, have been shown to be indicators of vocal deficits in other rodent disease models, such as aging and Parkinson Disease (Johnson et al., 2015). Therefore, detailed spectral analysis of FX mouse USVs could reveal vocal deficits and strengthen the neuroethological relevance of this model. To assess the spectral qualities of USVs in FX mice, a standard mating separation procedure was used (Johnson et al. 2011). Individual adult male mice were briefly exposed to an adult female mouse. Once the male expressed an interest in the female, the female was removed which elicited USVs from the male. The male USVs were recorded using an ultrasonic recording system (Avisoft, Germany) and analyzed using an existing custom MatLab program designed to detect, spectrally analyze, and classify mouse USVs (Holy and Guo, 2005). Our findings demonstrated that adult FX mice exhibited differences in various spectral properties and syllable formation compared to their wild-type counter parts. Furthermore, differences in USV production and quality in FX mice mirrored many vocal deficits seen in human FX vocalization patterns. This study provides a vital model for examining the biological factors mediating voice and speech abnormalities in Fragile X patients.
**Disclosures:** A. Belagodu: None. A. Johnson: None. R. Galvez: None.

**Poster**

**491. Fragile X Syndrome**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 491.02/E20

**Topic:** C.06. Developmental Disorders

**Support:** NIH Grant NS088776

**Title:** Fragile x knockout mice show alterations in activity levels and ultrasonic vocalization behaviors

**Authors:** S. NOLAN, C. REYNOLDS, G. SMITH, A. HOLLEY, M. VOLQUARDSEN, T. JEFFERSON, A. PANDIAN, T. SMITH, J. HUEBSCHMAN, *J. N. LUGO, JR; Psychology and Neurosci., Baylor Univ., Waco, TX

**Abstract:** Fragile X Syndrome (FXS) is a neurodevelopmental disorder caused by an overexpansion of a trinucleotide (CGG) repeat in the FMR1 gene coding for fragile x mental retardation protein. This disorder is characterized by intellectual disability as well as other behavioral abnormalities. In this study we examine ultrasonic vocalizations on postnatal days 10 and 12; locomotor activity, anxiety, and repetitive behavior in adult male and female FMR1 wild type and knockout (KO) mice using the FVB strain. We found that FMR1 knockout mice produce more ultrasonic vocalizations at the 60 kHz $t(1, 25) = 2.7, p < 0.05$; and at the 80 kHz level $t(1,25) = 2.3, p < 0.05$. They also produced a longer duration of 60 kHz calls at compared to the WT mice $t(1,25) = 2.4, p < 0.05$. When we examined the FMR1 knockout mice in adulthood we found several behavioral differences compared to wild type mice. We found a significant increase in total distance moved $t(1,31) = 3.7, p < 0.001$; increase in stereotypy time $t(1,31) = 2.32, p < 0.05$; and increase in rearing behavior $t(1,31) = 2.3, p < .05$ in the FXS knockout mice compared to wild type. We did not observe any significant alterations in anxiety through the elevated plus maze test or alterations in repetitive behavior through the marble burying test. Our results demonstrate that KO mice have altered communicative behavior during the early developmental periods, are hyperactive, and demonstrate some alterations in repetitive behavior. We are examining social behavior and learning and memory through other tests to examine the behavioral phenotype of the FMR1 knockout mice.
**Title:** Fmr1 knockout rats express hippocampus-dependent, spatial and episodic-like memory impairments

**Authors:** *A. ASIMINAS*,1,2, S. M. TILL,3,2, S. CHATTARJI,5,6, D. J. A. WYLLIE,3,2,6, P. C. KIND,3,2,6, E. R. WOOD,4,2;
1Patrick Wild Ctr., 2Ctr. for Integrative Physiol., 3Ctr. For Cognitive and Neural Systems, 4The Univ. of Edinburgh, Edinburgh, United Kingdom; 5Natl. Ctr. for Biol. Sci., Bangalore, India; 6Ctr. For Brain Develop. And Repair, Bangalore, India

**Abstract:** Fragile X syndrome (FXS) is the most common monogenic cause of intellectual disability and Autism Spectrum Disorder. It is, in most cases, caused by epigenetic silencing of the fragile X mental retardation gene (Fmr1), causing a loss of Fragile-X mental Retardation Protein (FMRP). The mouse model of FXS (Bakker et al., 1994) has been proven invaluable in FXS research so far, however the subtle and strain-specific behavioural phenotype has raised question over the validity of the model. The recent generation of a rat model of FXS paves the way for determining whether certain phenotypes are species or strain-specific or persist across mammalian species. We have recently shown that Fmr1−/y Sprague-Dawley (SD) rats exhibit a...
deficit in object-place-context task recognition (OPC) (Asiminas et al, SfN 2014. Poster 699.15). This hippocampus-dependent task requires the integration of object-location and contextual cues to form an episodic-like memory (Langston & Wood 2010). Here, we explore whether Fmr1-/-y Long-Evans-Hooded (LEH) rats exhibit the same deficit, to determine whether this phenotype persists across strains. LEH rats (n=16 Fmr1-/-y, n=16 Fmr1+/y) were tested in 4 spontaneous exploration tasks: novel object preference (NOP), object-context (OC), object-place (OP), and object-place-context (OPC). These tasks assessed the ability to discriminate novel from familiar objects, and novel from familiar object-context, object-place and object-place-context associations over a short (2 min) delay. Both groups showed significant memory in NOP, OC and OP tasks but only Fmr1+/y performed above chance in OPC task. Fmr1-/-y rats showed a decreased preference for novelty in OC and OP, and their ability to discriminate novel from familiar object-place-context (episodic-like) associations was significantly impaired. Spatial and object recognition memory were further assessed using object-displacement (OD) and NOP tasks at both short (2min) and long (24h) delay. Fmr1-/-y rats performed significantly worse than Fmr1+/y littermates in OD task at both delays and in NOR at the 24h delay. These data are consistent with your previous findings in SD rats and indicate that episodic (OPC) memory and spatial (OD) memory are impaired in Fmr1-/-y rats - across strains (LEH and SD). Deficits in these hippocampus-dependent tasks strongly suggest impaired hippocampal function which agrees with well-reported plasticity deficits in the mouse model of FXS and our recent work with SD Fmr1-/-y rats (Till et al, SfN 2013. Poster 810.10). This robust deficit in Object-place-context memory, across rat strains, provides an assay against which potential therapeutics could be tested.


491. Fragile X Syndrome

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 491.04/E22

Topic: C.06. Developmental Disorders

Support: Ministry of Science and Technology

Title: Study the fmr1 gene knock- out effects on the development of social- related behaviors by using zebrafish model
Authors: *M. T. HSU*¹, Y. J. WU¹, Y. L. YANG², K. T. LU¹;
¹Dept. of Life Sci., Natl. Taiwan Normal Univ., Taipei, Taiwan; ²Biochem. Sci. and Technol., Natl. Chia-Yi Univ., Chia-Yi, Taiwan

Abstract: Fragile X syndrome (FXS) is most generally hereditary form of human mental retardation. Previous research indicated that the most frequently cause of FXS was induced the abnormally function of the fragile X mental retardation 1 (fmr1) gene by triplet repeat expansion (CGG) mutation. The common symptoms of fragile X patients included learning disabilities, inattention, hyperactivity, anxiety, autistic behaviors, social impairments et al. In the present study, the fmr1 KO zebrafish model was applied for studying the behavioral effect of fmr1 gene, in order to clarify the relationship between the gene and the animal behaviors. We studied variable behavioral phenotypes, including shoaling behavior, social preference behavior, locomotor activity, and anxiety-like behavior. In summary, comparing with wild-type control, we found that 14 dpf fmr1 KO fish showing lessen total moving distance on locomotor activity. Conversely, there was more moving distance in the 28 dpf KO fish. Previous results showed the shoaling behavior would form in 14 dpf either wild-type or the fmr1 KO fish. However, the duration of closing to cluster of fishes would increase in the 28 dpf fmr1 KO fish. Then, according to another research, it showed that precocious development of social preference behavior in the 14 dpf fmr1 KO zebrafish, and the effect would sustain into adulthood. In addition, we wanted to investigate the factors of inducing above phenotypes, and we specially focused on the anxious expression. Therefore, we used the novel tank, a popular model for studying anxiety-like behavior, to analyze anxiety of the fmr1 KO fish. Results indicated that the fmr1 KO fish spent less time in upper half in the third 5 minutes interval, which displayed higher anxiety in the late larval stage of the fmr1 loss-off function. In conclusion, our results indicated that having highly relationship between the functions of fmr1 and the development of social-relative behaviors. Furthermore, the elevated anxiety status may account for the abnormal social-relative behaviors in fmr1 KO fish. We suggested that fmr1 KO zebrafish is an idea model for pre-clinical drug screening of autism.

Disclosures: M.T. Hsu: None. Y.J. Wu: None. Y.L. Yang: None. K.T. Lu: None.
**Support:** Brain Research Foundation, Finland
Academy of Finland

**Title:** Effects of reduced levels of BDNF expression on the differentiation of neural progenitors in Fragile X syndrome

**Authors:** *V. S. ACHUTA, G. TURCONI, M. CASTREN;
Physiol. Dept., Univ. of Helsinki, Helsinki, Finland

**Abstract:** Fragile X syndrome (FXS) is the most common cause of inherited intellectual disability and a well characterized form of autism spectrum disorder. A triplet repeat expansion in the *FMR1* gene leads to transcriptional silencing and the absence of FMR1 protein (FMRP) in FXS. FMRP is a RNA binding protein essential for maturation and function of synapses and neuronal networks. Neural progenitor differentiation is altered in the absence of FMRP. Studies of *Fmr1* knockout (KO) mice, a mouse model of FXS, have revealed alterations of glutamate and brain-derived neurotropic factor (BDNF)/TrkB signaling in FXS brain. BDNF signaling is also affected in FMRP-deficient progenitors. We compared the differentiation of FXS and wild-type neural progenitors and examined the role of BDNF in the alterations of the differentiation of neuronal cells in FXS neurospheres. Mouse cortical progenitors were generated from *Fmr1* KO mice and double transgenic mice deficient of both FMRP and BDNF. Human neural progenitors were differentiated from induced pluripotent stem (iPS) cell lines reprogrammed from somatic cells of individuals with a triplet repeat mutation in the *FMR1* gene and characterized by pluripotency markers, karyotyping, and genetic mutation analysis. Cell imaging and intracellular calcium recordings with Fura-2 were used to examine the cell kinetics and functional responses to glutamate receptors at different stages of neuronal differentiation. We found alterations in the responses to activation of metabotropic glutamate receptors by (S)-3,5 dihydroxyphenylglycine (DHPG) and ionotropic glutamate receptors by kainate and N-methyl-D-aspartate (NMDA) in human and mouse FXS neural progenitors. The reduced BDNF expression modified the maturation of glutamate responses and cell migration in mouse progenitors lacking FMRP.

Altogether, our results show that BDNF contributes to the alterations of transmitter plasticity in FXS.

**Disclosures:** V.S. Achuta: None. G. Turconi: None. M. Castren: None.

**Poster**

491. Fragile X Syndrome

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 491.06/E24
Deficits in adult neurogenesis in Fragile X Syndrome

**Authors:** *C. DAVIS¹, M. DEMARS², J. LARSON³, O. LAZAROV²;
²Anat. and Cell Biol., ³Psychiatry, ¹Univ. of Illinois At Chicago, Chicago, IL

**Abstract:** Fragile X Syndrome (FXS) is the most prevalent inheritable form of mental retardation in humans and is the most common known genetic cause of autism. FXS is an X-chromosome-linked disorder characterized by hypermethylation of a CGG repeat expansion in the promoter region of the Fmr1 gene causing gene silencing and subsequently a deficiency of the fragile X mental retardation protein (FMRP). Studies have demonstrated impaired synaptic function and plasticity in the brain in the absence of FMRP. However, the mechanism underlying mental retardation is not fully elucidated. Adult neurogenesis is implicated in learning and memory and in brain plasticity. Neurogenesis takes place in the subgranular layer (SGL) of the dentate gyrus (DG) of the hippocampus and the subventricular zone (SVZ). Here, we examined whether deletion of fmr-1 affects neurogenesis in adult Fmr1 knockout (Fmr1-KO) mice. We show that the number of fast-proliferating cells is dramatically reduced in the SVZ and the SGL in Fmr1-KO mice when compared to wild type littermates. Additionally, the rate of survival of neuroblasts in the SGL was dramatically compromised. Finally, we show that these impairments result in significantly fewer mature neurons in the DG of the Fmr1-KO mice. Taken together, these results suggest that compromised neurogenesis may cause reduced plasticity and may underlie learning deficits in FXS.

**Disclosures:** C. Davis: None. M. Demars: None. J. Larson: None. O. Lazarov: None.

**Poster**

491. Fragile X Syndrome

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#: 491.07/E25

**Topic:** C.06. Developmental Disorders

**Support:** The Azrieli Neurodevelopmental Research Program
Title: Postnatal hippocampal neural precursor cells show an altered cell cycle profile in the Fragile X mouse

Authors: *M. SOURIAL, H. LIANG, L. C. DOERING; McMaster Univ., Hamilton, ON, Canada

Abstract: Fragile X Syndrome (FXS) is the leading single gene cause of autism and inherited intellectual impairment. It results from the epigenetic transcriptional silencing of the Fragile X Mental Retardation 1 (FMR1) gene, and the consequent loss of expression of the Fragile X Mental Retardation Protein (FMRP). FMRP plays an important role in synaptic plasticity and has been implicated in neural precursor cell (NPC) proliferation and differentiation. We have shown that hippocampal NPCs isolated from Fmr1 knockout (KO) mice have different expression profiles of SOX2, Nestin, and Ki67 in vitro compared to their wild type (WT) counterparts, which indicated an altered proliferative capacity of Fmr1 KO NPCs. Thus, the current experiments assess the in vivo expression of NPC markers and the cell cycle profile in the hippocampus of postnatal Fmr1 KO mice. Postnatal day 4 (P4) mouse hippocampi were dissociated and fixed with ethanol and the DNA was stained with propidium iodide to analyze cell cycle phases. Preliminary results indicate that hippocampi from Fmr1 KO mice show an increased proportion of cells in the S and G2/M phases compared to WT mice. Based on the fact that cells with low to medium levels of CD133 and an absence of CD24 generate neurospheres (spherical clusters of NPCs in vitro), continuing experiments using flow cytometry comparing NPC expression of CD15, CD24, and CD133 will help to elucidate the effects of FMRP on the proliferative capacities of NPCs. In line with our previous findings, our current results demonstrate that the proliferation of NPCs in the hippocampus is significantly affected in FXS.

Disclosures: M. Sourial: None. H. Liang: None. L.C. Doering: None.

Poster

491. Fragile X Syndrome

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 491.08/E26

Topic: C.06. Developmental Disorders

Support: FRAXA Program Grant

NFXF Summer Undergraduate Summer Fellowship
Title: Auditory stimulation differentially deactivates ERK in the amygdala of juvenile FMR1 KO mice susceptible to audiogenic seizure (AGS): effect of GABA(A) modulation

Authors: M. H. DAVENPORT1,2, A. A. ASHWORTH1, M. S. STEGMAN1, C. A. ERICKSON1, *T. L. SCHAEFER1;

Abstract: Fragile X Syndrome (FXS) is the most common single gene cause of autism spectrum disorder and intellectual disability. FXS is caused by a CGG repeat expansion in the 5' UTR of the Fragile X Mental Retardation 1 (FMR1) gene that leads to loss of expression of the Fragile X Mental Retardation Protein (FMRP). Patients are characterized by a range of intellectual, behavioral, and physical impairments, including increased risk of seizure, hypersensitivity to auditory stimuli, and increased fear and anxiety. These phenotypes are recapitulated in the Fmr1 knock out (KO) mouse model of FXS. The immediate early gene, extracellular signal-regulated kinase (ERK1/2), has long been implicated in FXS pathophysiology and basal levels of ERK1/2 activation have been shown to be increased in brain regions important for fear processing and cognition. In the context of FXS, ERK signaling is thought to be a convergence point for a variety of cellular signaling cascades and is used as an indication of aberrant cellular signaling in both mouse and human studies. In normal behaving rodents, cell-type and regionally specific coordination of activation and deactivation of ERK is known to be required for appropriate behavior. Here, we show an increased number of immuno-positive cells expressing activated ERK in the central amygdala (CeA; output center of the amygdala) with no differences in sporadic expression in the lateral amygdala (LA; main input center of the amygdala) during basal conditions in juvenile Fmr1 KO mice. However, immediately following an audiogenic seizure (AGS) paradigm, ERK1/2 phosphorylation is dramatically reduced in the CeA and increased in the LA of Fmr1 KO mice that experience tonic/clonic seizure activity compared to both WT and Fmr1 KO mice that do not seize. To reduce hyper-excitability in the brain of FXS mice, we acutely treated juvenile Fmr1 KO mice with a GABA(A) alpha 2, 3 agonist (AZD7325). AZD7325 treatment reduced the seizure susceptibility of Fmr1 KO mice in a dose dependent manner. Even in this treatment experiment, mice who exhibited seizure activity regardless of dose still demonstrated ERK deactivation in the CeA and increased activation in the LA. This indicates that region specific alterations in ERK activity within the amygdala are concomitant with seizure activity and further demonstrates that ERK phosphorylation change upon sensory stimulation plays an important role in FXS associated signaling and behavioral deficits. We are currently working to determine the specific cell types that express activated ERK in the CeA and LA at both basal and following AGS to better understand the circuitry that is disrupted in the developing FXS brain.

Disclosures: M.H. Davenport: None. A.A. Ashworth: None. M.S. Stegman: None. C.A. Erickson: None. T.L. Schaefer: 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.; Fraxa
Research Foundation Grant. C. Other Research Support (receipt of drugs, supplies, equipment or
other in-kind support); Astra Zeneca.

**Poster**

**491. Fragile X Syndrome**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 491.09/E27

**Topic:** C.06. Developmental Disorders

**Support:** Lundbeck Foundation

Danish MRC

**Title:** Nmdar nr2a and nr2b specific pke-dependent regulation of mglur is defective in the fragile
x syndrome mouse model

**Authors:** *T. G. BANKE, A. K. H. TOFT;
Aarhus Univ., Aarhus, Denmark

**Abstract:** The Fragile X Syndrome (FXS) animal model, the Fmr1 knock-out (KO) mouse, has
demonstrated an increased mGluR5-mediated long-term depression (LTD). However,
surprisingly little information exists about other ion channels/receptors and their effects on FXS,
including NMDA receptors (NMDAR). Here we focus on the two main types of hippocampal
LTD: (1) NMDA-dependent, and (2) mGluR-dependent but NMDA-independent, at different
developmental stages. We applied the mGluR agonist DHPG in the presence or absence of the
NMDAR competitive antagonist, APV, hereby unmasking the NMDAR component in this
process. As reported by several labs, using the field potential recording technique in acute slices
from young mice (P30-40), application of DHPG, in the presence of APV, induced more LTD in
the KO mouse than in the control mouse (41+/−5%, n=7 vs. 21+/−4%, n=5, P<0.05). In the
absence of APV, DHPG induced the same amount of LTD in KO vs WT (39+/−6%, n=11 vs.
33+/−5%, n=7, P=0.2), suggesting that NMDARs play an insignificant role in DHPG-induced
LTD in both the KO and WT mouse at this young developmental stage. As with young mice, in
slices from mature WT mice (P60-80), there was no significant difference in DHPG-induced
LTD in the absence of APV (28+/−6%, n=12) or in the presence of APV (32+/−5%, n=8).
Equivalent LTD was obtained in the KO mouse in the presence of APV (36+/−6%, n=9). This
was in sharp contrast to recordings from KO mice where we found in the absence of APV, no or
very little LTD (7+/−5%, n=15). Similar results were observed when mature KO slices were
preincubated with the protein kinase C activator (PMA), or inactivator (chelerythrine), suggesting that NMDAR activation leads to PKC regulation of mGluR-LTD. Surprisingly, preincubation WT with the NR2B specific NMDAR inhibitor CP-101.606 completely blocked DHPG-induced LTD. This blockage was reversed by either preincubation with PMA or by co-application of the NR2A antagonist TCN-201. In contrast, application of TCN-201 alone had no apparent effects. Our data suggest a model where NMDARs regulate mGluR-LTD through regulation of PKC. Furthermore, in this model it appears that NR2B activation stimulates PKC, while NR2A activation halts or reverses this effect. In addition, in the KO mice, the coupling between specific NMDAR subunits and mGluR-LTD activity through PKC seems defective in an age-dependent manner. These findings suggest strong developmental involvement of NMDARs in the pathophysiology of FXS and highlight a novel potential path for FXS treatment.

Disclosures: T.G. Banke: None. A.K.H. Toft: None.

Poster

491. Fragile X Syndrome

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 491.10/E28

Topic: C.06. Developmental Disorders

Support: MH097093

Title: Network-level plasticity of Up states and evoked activity in Fragile X Syndrome circuits

Authors: *H. MOTANIS, D. BUONOMANO;
Dept. of Neurobio. and Psycholology, and Integrative center for learning, UCLA, Los Angeles, CA

Abstract: Since the generation of the first mouse model of FXS a broad range of neurophysiological phenotypes have been reported. However, it remains unclear which phenotypes are causally related to the cognitive deficits associated with FXS. Indeed, because many of these phenotypes are known to be modulated by experience, a confounding factor in the interpretation of many studies is whether some phenotypes are an indirect consequence of abnormal development or experience. In vitro developmental studies provide one approach towards addressing these confounds since observed phenotypes are more likely to arise from the genotype and not from abnormal experience or development. We previously found a significant developmental delay in the emergence of spontaneous activity and Up states in FXS circuits, demonstrating that developmental delays characteristic of FXS are recapitulated during in vitro
development, and that Up state abnormalities are likely direct consequence of the disease. Here we explore activity-dependent modulation of evoked and spontaneous activity in Fmr1-/-y circuits. We used chronic optogenetic stimulation of organotypic slices to emulate a developmental increase in externally driven activity, and induce homeostatic plasticity of spontaneous and evoked network activity. After transfection AAV5-CaMKIIa-ChR2(H134R)-EYFP, slices from WT and Fmr1-/-y mice were optically stimulated for 2 days. Whole-cell recordings were made from layer II/III pyramidal neurons. Chronic optical stimulation of both WT and Fmr1-/-y circuits resulted in a significant reduction of spontaneous Up states: Up state frequency were significantly reduced following stimulation (F2,46=25.42, p<10^-7). And there was no genotype difference in the homeostatic decrease in Up states, indicating that Fmr1-/-y circuits exhibit normal homeostatic plasticity. Chronic optical stimulation also induced a significant decrease of evoked EPSP strength (F1,41=9.28, p<0.005), as measured by the asymptotic strength of the evoked EPSP, and two-way ANOVA revealed a significant genotype effect (F1,41=8.08, p<0.01). Importantly, the EPSP strength in unstimulated Fmr1-/-y circuits was smaller compared to unstimulated WT circuits (p<0.05) indicating abnormal baseline response in Fmr1-/-y circuits. These results indicate that while activity induced plasticity of spontaneous Up states is normal in "mature" cortical Fmr1 circuits, the baseline EPSP strength and plasticity of EPSPs is not. These results provide the first steps towards using cortical organotypic cultures as a reduced system to unravel the circuit level mechanisms that underlie the learning deficits that characterize FXS.

Disclosures: H. Motanis: None. D. Buonomano: None.
Title: Altered number of neural population activity patterns in Fragile-X mice

Authors: *C. O’DONNELL1, J. T. GONCALVES1, C. PORTERA-CAILLIAU2, T. J. SEJNOWSKI1,3;
1Salk Inst. For Biol. Studies, La Jolla, CA; 2Departments of Neurol. and Neurobio., UCLA, Los Angeles, CA; 3Div. of Biol. Sci., UCSD, La Jolla, CA

Abstract: Brain disorders such as Fragile X syndrome (FXS), autism and schizophrenia have been associated with different changes in neural function, such as an imbalance in excitation and inhibition, hyperexcitability, and altered synaptic connectivity. Why or how these particular changes would be detrimental for neural coding remains unknown. Here we test the hypothesis that these cellular changes affect information processing by shifting the typical number of activity patterns used by the circuit. We analyzed spontaneous activity of neural populations in somatosensory cortex recorded *in vivo* using two-photon calcium imaging in both wild-type and Fmr1 knockout mice, a model for FXS. We developed a new statistical model for the probability distribution of all $2^N$ possible neural population activity patterns that required only $N^2$ parameters, where N is the number of neurons. Using this analysis method, we found that even subtle alterations in neural firing rates and correlations in Fmr1 knockout animals, relative to wild-type, across development lead to dramatic shifts in the typical number of circuit activity patterns. Adolescent Fmr1 knockout mice showed fewer neural activity patterns than wild-type, while the opposite was true in adult mice. Using a computational model of mouse layer 2/3 somatosensory cortex, we explored the contributions of the underlying circuit components, leading to two main conclusions: first, there are multiple non-exclusive neurobiological routes to altered circuit dimensionality. Second, the type of parameter changes needed to reverse the FXS deficits is qualitatively different in adolescent versus adult mice. If also true in humans, this result suggests that a pharmaceutical intervention that can successfully reverse symptoms in adults with FXS might prove ineffective or possibly even exacerbate symptoms in children with this disorder. Altogether, our findings show how relatively small changes in neural circuit parameters in brain disorders can have dramatic consequences for information processing.


Poster

491. Fragile X Syndrome

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM
Title: Regulation of protein synthesis by Bcl-xL and its potential application in the treatment of Fragile X

Authors: *P. LICZNERSKI*, P. MIRANDA, H.-A. PARK, M. BROWN, L. K. KACZMAREK, R. J. LEVY, E. A. JONAS;

Abstract: We have found previously that Bcl-xL, a member of the Bcl-2 family of proteins, acutely and chronically enhances synaptic connectivity in multiple ways. Bcl-xL is necessary for certain forms of synaptic plasticity, alters mitochondrial positioning and metabolism in the synapse and directly affects vesicle trafficking. Our studies suggest that Bcl-xL could play a role in aberrant development of synapses observed in Fragile X syndrome (FXS). Indeed, studies in two different Fmr1 mutant strains showed increased expression of Bcl-xL. So far, we have found that: 1) Inhibition of Bcl-xL by the small molecule inhibitor ABT-737 produces a marked enhancement of protein translation. 2) The increased rate of protein translation produced by ABT-737 is comparable to or greater than that produced by FMRP knock out. 3) The increase in protein translation produced by ABT-737 is occluded in the Fmr1-- mouse neurons, suggesting that inhibition of Bcl-xL or genetic deletion of Fmr1 both produce an effect on protein translation perhaps through the same mechanism. The present study examines the gene pool translationally-regulated by Bcl-xL and addresses the possibility of Bcl-xL to regulate protein translation by modulating the cross-talk between mitochondria and endoplasmic reticulum (ER) via Mitochondria-Associated Membranes (MAMs).

Title: HDAC inhibition rescues cognitive impairments in a *Drosophila* Fragile X model

Authors: *S. M. McBride*¹, B. Shoenfeld¹, C. Choi¹, A. Bell¹, P. Hinchey², M. Kollaros², A. Terlizzi², N. Ferrick², D. Liebelt², D. Emerson¹, A. Rostain¹, S. Siegel¹, T. McDonald², T. Jongens¹; ¹Univ. of Pennsylvania, Philadelphia, PA; ²Albert Einstein Col. of Med. of Yeshiva Univ., Bronx, NY

Abstract: Fragile X Syndrome is caused by loss of FMR1 gene activity and is the most commonly inherited form of cognitive impairment and autism. Patients with this disorder also suffer from hyperactivity, attention deficit disorder, irritability, sleep problems and have noted neuro-anatomical defects. A *Drosophila* fragile X model, based on loss of dfmr1 function, displays several relevant phenotypes, including defects in circadian regulation, social interaction (with peers and in naïve courtship), memory and morphology of some neurons in the brain. Here we show that the memory deficits displayed by dfmr1 mutants can be rescued by reducing histone deacetylase activity. We demonstrate that pharmacologic treatments with trichostatin A or sodium butyrate given in development and in adulthood can rescue immediate recall memory, short term memory and long term memory in fragile X flies. Our results indicate that histone deacetylase inhibitors can rescue cognitive phenotypes displayed by the *Drosophila* fragile X model, and thereby revealing another pathway that can be targeted by new and current drugs to treat fragile X patients.

Poster

491. Fragile X Syndrome

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 491.14/E32

Topic: C.06. Developmental Disorders

Support: Brain Canada
The Azrieli Neurodevelopment Research Program
FRAXA Research Foundation

Title: Altered cellular physiology of astrocytes in a model of fragile x syndrome

Authors: *A. L. SCOTT¹, C. CHENG², L. C. DOERING¹;
¹Dept. of Pathology an Mol. Med., ²McMaster Univ., Hamilton, ON, Canada

Abstract: Neural communication and the intricate choreography of signals required for the formation and preservation of neural connections is heavily dependent on reciprocal neuronal and glial interactions. Astrocytes are key participants in neurodevelopmental processes and defects to astrocyte signaling are implicated in many disease states characterized by abnormal neural circuitry, such as Fragile X Syndrome (FXS). In FXS, the loss of the Fragile X mental retardation protein (FMRP) expression from astrocytes in particular is associated with delayed dendrite maturation and improper synapse formation. These findings emphasize the importance of astrocyte-derived signals to the establishment of neuronal connections and illustrate the negative consequences an imbalance to these signals can cause. During development astrocytes release a wide range of neurotransmitters; however, the use of ATP is one of the predominant means of communication between astrocytes and neurons within the CNS. ATP is a fast, excitatory neurotransmitter known to act on astrocytes, modulate glio-neuronal transmission, and in this way regulate synaptic function. Given the integral role of ATP, and its various metabolites (ADP, AMP, adenosine), to the regulation of synaptic development and function, we compared the physiological responses of astrocytes isolated from either post-natal wild-type (FMRP+/+) mice or from transgenic fmr1 knockout (FMRP−/−) mice to exogenous ATP application. The ratiometric analysis of intracellular calcium levels measured with the calcium indicator Fura2-AM, revealed a significantly greater flux of intracellular calcium in FMRP−/− astrocytes in response to ATP than observed in FMRP+/+ astrocytes. The differences in calcium responses suggest that the sensitivity of the FMRP−/− astrocytes to ATP is abnormally enhanced, and may underlie atypical neural communication present during development in FXS. Future analysis of the effects on astrocyte-neuron purinergic signaling in the FMRP−/− model will help elucidate the
potential role these signals play in FXS. This work is supported by Brain Canada and the Azrieli Neurodevelopmental Research Program. A.L. Scott is a FRAXA postdoctoral fellow.


Poster
491. Fragile X Syndrome

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 491.15/E33

Topic: C.06. Developmental Disorders

Support: Brain Canada Azrieli Neurodevelopmental Research Program

Title: Reduced cortical expression of astrocyte-secreted glypicans 4 and 6 in the fragile X mouse model

Authors: *J. WALLINGFORD, L. C. DOERING;
Pathology and Mol. Med., McMaster Univ., Hamilton, ON, Canada

Abstract: Astrocytes play an important role in the development and maintenance of proper neural circuitry within the CNS. Many neurodevelopmental disorders have been associated with aberrant astrocyte signalling and function, including fragile X syndrome (FXS). FXS, the most common inherited single gene cause of autism, is characterized by a deficiency in fragile X mental retardation protein (FMRP) via the silencing of the fragile X mental retardation 1 (FMR1) gene. FMRP, which is expressed by both astrocytes and neurons, acts as an important regulator of synaptic development through its ability to bind, transport, and regulate the local translation of particular synaptic mRNAs. Using a Fragile X knockout (KO) mouse model we examined whether there are altered expression levels of the astrocyte-secreted factors SPARC-like 1 (SC1; also known as Hevin) and glypicans 4 and 6 (GPC 4/6), corresponding to FMRP deficient astrocytes. These factors play important and complementary roles during excitatory synapse development. While the matricellular protein SC1, a known target of FMRP, promotes the structural development of synapses, they are postsynaptically silent due to their lack of postsynaptic a-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors (AMPARs). GPC 4/6, members of the heparin sulphate proteoglycan family, are involved in recruiting AMPARs to the postsynaptic membrane surface, inciting postsynaptic function. Cortical tissue from KO and WT mice (n=4/group) was collected at postnatal day 19 and analyzed for SC1 and
GPC 4/6 expression via Western blotting. Although we found no difference in cortical levels of SC1 between the groups, GPC 4/6 was reduced in the cortex of KO mice compared to controls. Further experiments will examine SC1 and GPC 4/6 levels in the hippocampus and at additional developmental time-points. These preliminary results suggest that reduced levels of GPC 4/6 may contribute to improper synapse development in FXS. This work is supported by Brain Canada and the Azrieli Neurodevelopmental Research Program.

Disclosures: J. Wallingford: None. L.C. Doering: None.

Poster

491. Fragile X Syndrome

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 491.16/E34

Topic: C.06. Developmental Disorders

Support: Brain Canada

Azrieli Neurodevelopmental Research Program

Title: Developmental reductions in thrombospondin-1 expressing astrocytes characterize the fragile x mouse model

Authors: *C. CHENG, S. K. M. LAU, J. WALLINGFORD, L. C. DOERING; McMaster Univ., Hamilton, ON, Canada

Abstract: Astrocytes are key participants in many aspects of brain development and function. Defects in astrocyte signaling are implicated in neurodevelopmental disorders characterized by abnormal neural circuitry, such as Fragile X syndrome (FXS). FXS is the most common inherited form of intellectual disability and autism spectrum disorders. FXS is caused by silencing of the fragile X mental retardation 1 (FMR1) gene, which results in the loss of the fragile X mental retardation protein (FMRP). Thrombospondin-1 (TSP-1) is an astrocyte-secreted protein that plays a critical role in regulating synapse formation and integrity during development. TSP-1 is expressed in immature astrocytes and its expression peaks during the first postnatal week in mice, which coincides with the expression of FMRP. Our earlier findings revealed that FXS astrocytes express a deficiency in cellular and secreted TSP-1, and affect synapse and spine development in vitro. In this study, we examine the developmental trajectory of TSP-1 expression in cortical astrocytes of fmr1 wild-type (WT) and knockout (KO) mice at 7, 14 and 21 days in vitro (DIV). We assessed TSP-1 expression by semi-quantitative
immunocytochemistry in cells co-labeled with the astrocyte markers, glial fibrillary acidic protein (GFAP) and aldehyde dehydrogenase 1 family, member L1 (ALDH1L1). Our results showed the highest expression of TSP-1 positive astrocytes in both WT and fmr1-KO mice to be at 7 DIV. The expression of TSP-1 subsequently declined in both WT and fmr1-KO astrocytes at later time points. Significant differences between WT and fmr1-KO astrocytes in TSP-1 expression were observed at 14 and 21 DIV. These results indicate that defects in the secretion of astrocyte-specific molecules during a critical window of development contribute to FXS neurobiology. This work is supported by Brain Canada and the Azrieli Neurodevelopmental Research Program.


Poster

491. Fragile X Syndrome

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 491.17/E35

Topic: C.06. Developmental Disorders

Support: Autism Science Foundation

FRAXA Research Foundation

Title: Astroglial fragile X mental retardation protein contributes to pathogenesis of Fragile X Syndrome

Authors: *H. HIGASHIMORI1, C. SCHIN2, Y. YANG2;
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Abstract: Fragile mental retardation protein (FMRP) is enriched in neurons and has been characterized as an important translational repressor in neurons; however, a selective deletion of FMRP in a significant number of cortical and hippocampal neurons showed only limited FXS related phenotypes. Here we aim to investigate what role the astrocyte specific loss of FMRP plays in FXS pathophysiology. We generated the inducible astrocyte specific fmr1 conditional knock out (astro-fmr1-cKO) mice to investigate the pathogenic role of the selective deletion of astroglial FMRP in FXS. We found that the selective loss of astroglial FMRP particularly contributed to the reduction of GLT1 expression and function to a level similar to that of fmr1-/- animals we had previously observed. Moreover, the loss of astroglial GLT1 function in the astro-
fmr1-cKO mice was sufficient to increase the firing rate of neocortical layer 5 somatosensory pyramidal neurons by exposure to low doses of dihydrokainic acid (DHK), a GLT1 specific inhibitor. DHK did not have this effect on cortical neurons from control animals. Our findings thereby demonstrate that GLT1 dysfunction can reduce glutamate uptake in the astro-fmr1-cKO mice, causing neuronal cells to become hyper-excitible. We also found that the enhanced cortical neuronal excitability is due to the persistent activation of NMDA receptors by glutamate. This provides an increased tonic excitatory drive to somatosensory pyramidal neurons. We also observed immature synaptic morphology and increased cortical protein synthesis in the astro-fmr1-cKO mice, which recapitulates the FXS related phenotype. To further investigate the role of astrocytic FMRP function, we generated the inducible astrocyte specific fmr1 conditional restoration mice (astro-fmr1-cON). Inducing expression of FMRP in Astro-fmr1-cON mice restored cortical GLT1 expression, tonic excitatory drive and enhanced NMDA activation in the somatosensory cortex. This study is the first to address whether the dysfunction of the astroglial FMRP and glutamate transporter GLT1 contributes to the development of FXS. In the future, we will perform various behavioral assays on astrocytic conditional knockout and restoration mice.

Disclosures: H. Higashimori: None. C. Schin: None. Y. Yang: None.

Poster

491. Fragile X Syndrome

Location: Hall A

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Topic: C.06. Developmental Disorders

Support: FRAXA Foundation

Telethon Foundation Grant GGP13145

Title: Effect of 5-HT7 receptor activation on dendritic spines in wild-type and Fmr1 knockout mice

Authors: M. SPATUZZA¹, S. D'ANTONI¹, G. LA QUATRA¹,², C. M. BONACCORSO³, M. LEOPOLDO⁴, *L. CIRANNA², M. V. CATANIA¹,²

¹Inst. of Neurolog. Sci. (ISN), Natl. Res. Council (CNR), Catania, Italy; ²Univ. of Catania, Catania, Italy; ³Lab. of Neurobio., IRCCS Oasi Maria Santissima, Troina (EN), Italy; ⁴Univ. of Bari, Department of Pharmacy, Italy
Abstract: Serotonin (5-HT) is an important neurotransmitter expressed in different brain areas. 5-HT is involved in remodeling brain structures and circuits during development, through the modulation of several processes including neural cell proliferation, migration and differentiation, neurite outgrowth, axon guidance and synaptogenesis, control of dendritic spine shape and density. Recently, we have shown that activation of 5-HT7 receptors reverses metabotropic glutamate receptor-mediated long-term depression (mGluR-LTD) in wild-type (WT) and Fmr1 knockout (KO) mice (Costa et al., Biol Psychiatry. 2012, 72(11):924-33), a mouse model of Fragile X syndrome (FXS), which is characterized by an abnormally enhanced mGluR-LTD. Here, we extended our study to the examination of dendritic spine morphology and density, which are abnormal in FXS. We studied the expression of 5-HT7 receptors in synaptic plasma membranes (SPMs) prepared from cortices and hippocampi of WT and Fmr1 KO mice, and found that in SPMs of both regions 5-HT7 receptors are present in the post-synaptic fraction in both genotypes. We tested the effects of an acute systemic administration of the compound LP-211 (3 mg/Kg), a potent, selective and brain-permeant 5-HT7 receptor agonist in WT and Fmr1 KO mice. We evaluated dendritic spine density and morphology in the cortex and the CA1-CA3 areas of hippocampus by analyzing confocal microscopy images from slices labeled with the lipophilic tracer DiI. Our results indicate an increased spine density in both cortex and hippocampus in Fmr1 KO mice, which was not detected after LP-211 administration in both brain regions. Interestingly, the reduction of spine density in LP-211-treated Fmr1 KO mice affected thin spines. Our data suggest that in vivo administration of a selective agonist of 5-HT7 receptors results in the correction of increased spine density, a hallmark feature of FXS, suggesting that targeting of 5-HT7 receptors might be considered as a new therapeutic strategy for FXS.


Poster
491. Fragile X Syndrome

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 491.19/E37

Topic: C.06. Developmental Disorders

Support: Fraxa foundation

Title: NKCC1 inhibitor rectifies critical period synaptic development and plasticity in Fragile X mice
Abstract: Disruptions in GABA signaling are prevalent in Fragile X syndrome (FXS) and may contribute to the alterations in synaptic development and hyperexcitability in the cortex of the mouse model of FXS (Fmr1−/y). We have recently determined that there is a delay in the normal polarity switch in GABA responses in cortical neurons during the critical period in FXS mice. This delay is accompanied by elevated expression of the juvenile chloride cotransporter, NKCC1. Here we have tested whether postnatal administration of the NKCC1 inhibitor bumetanide can correct the dysregulated progression of GABA reversal potential (EGABA), and whether this rescues known synaptic alterations in the developing somatosensory cortex. Fmr1−/y and littermate control (Fmr1+/y) pups were injected daily with bumetanide (0.2mg/kg) or saline solution beginning at postnatal day P0. Perforated patch-clamp recordings were made to measure EGABA in layer 4 cortical neurons in acute slices at postnatal day P10. In saline injected Fmr1−/y mice, EGABA remained relatively depolarized at -54 ± 7 mV (n=14). In contrast, recordings from bumetanide injected Fmr1−/y mice EGABA was hyperpolarized -82 ± 9 mV (n = 8, P < 0.05) and similar to EGABA in vehicle treated littermate controls. We next determined whether chronic bumetanide injection could also rectify the delay in synaptic development and critical period plasticity. EPSCs were evoked with a bipolar electrode placed in the ventrobasal thalamus and the amplitudes of the NMDAR and AMPAR components measured to calculate the NMDAR/AMPAR (N/A) current ratio. In saline injected Fmr1−/y, N/A ratio was 1.05 ± 0.11 (n = 12) at postnatal day P7. In contrast, bumetanide treatment reduced the N/A ratio to 0.76 ± 0.06 (n = 12 P < 0.05) in Fmr1−/y, which is similar to that in vehicle treated controls. In a separate cohort of animals we determined whether bumetanide could also rescue critical period synaptic plasticity alterations. This NMDAR dependent long-term potentiation, that is present at early postnatal times and absent at the closure of the critical period (P7), persists to a later developmental age in Fmr1−/y mice. In saline treated Fmr1−/y at P7, 1 Hz presynaptic stimulation paired with postsynaptic depolarization lead to a 130 ± 8 % (n = 12) potentiation of EPSCs. In recordings from bumetanide treated Fmr1−/y animals no significant potentiation was observed at this developmental stage (94 ± 7 %, n = 8, P < 0.05) similar to vehicle treated Fmr1+/y controls. Taken together our results demonstrate that chronic treatment with an NKCC1 inhibitor during the postnatal critical period can rectify synaptic development and plasticity phenotypes in the cortex of the FXS mouse model.

Disclosures: Q. He: A. Employment/Salary (full or part-time); Northwestern University Feinberg School of Medicine. C. Piochon: None. A. Contractor: None.
491. Fragile X Syndrome

**Location:** Hall A

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**Program#/Poster#:** 491.20/E38

**Topic:** C.06. Developmental Disorders

**Support:** NIH Grant 2T32MH067564

**Title:** Gaba signaling in adult-born neurons in fragile x syndrome

**Authors:** *C. REMMERS*¹, A. CONTRACTOR¹²; ¹Dept. of Physiol., Northwestern Univ. Feinberg Sch. of Med., Chicago, IL; ²Dept. of Neurobio., Northwestern Univ. Weinberg Col. of Arts and Sci., Chicago, IL

**Abstract:** Fragile X syndrome (FXS) is the most common form of inherited human mental retardation and also the single most common known cause of autism. FXS most often results from the expansion of a CGG repeat sequence in the 5’ untranslated region of the gene, which leads to loss of expression of the Fragile X mental retardation protein (FMRP). Loss of FMRP in mutant mice (Fmr1 KO) leads to multiple phenotypic alterations including impaired learning and memory and alterations in synaptic plasticity. One important regulator of neuronal development that is disrupted in FXS is the inhibitory neurotransmitter GABA. During early development GABA is excitatory, and depolarizing GABA has trophic effects on proliferation, differentiation, dendritic morphogenesis, and synaptogenesis. These effects may be mediated by synaptic responses or by tonic activation of extrasynaptic GABA receptors. Recent studies in our laboratory have demonstrated that the switch in GABA polarity from depolarizing to hyperpolarizing responses is delayed in the cortex of perinatal Fmr1 KO mice. This delay is accompanied by elevated expression of the chloride co-transporter that is primarily responsible for maintaining the depolarized reversal potential for chloride ions, NKCC1. In the adult brain, depolarizing GABA is critical for the survival and development of adult-born neurons in the hippocampus. Previous studies have clearly demonstrated a role for FMRP in adult neurogenesis in the dentate gyrus; however, the mechanisms of this impairment are not known and the progression of the developmental switch in depolarizing GABA in adult-born neurons has not been assessed in the mouse model of FXS. Therefore there is a strong possibility that the impaired neurogenesis and related learning deficits are a result of an alteration in maturation of the GABA polarity switch. In this study we have used a retroviral labeling technique to birthdate newborn dentate granule cells in Fmr1 KO mice. Using perforated patch recordings from retrovirus labeled neurons we are able to determine whether the time course of depolarizing GABA in adult-born neurons is disrupted in Fmr1 KO mice. In addition, recording of tonic currents will determine whether there are changes in the driving force through GABA channels,
which could influence excitability of adult born neurons. Taken together these studies will be the first to determine whether alterations in GABA polarity can influence the functional properties of newborn neurons in FXS mice.

Disclosures: C. Remmers: None. A. Contractor: None.

Poster

491. Fragile X Syndrome

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 491.21/E39

Topic: C.06. Developmental Disorders

Title: Quantification of FMRP in human and mouse tissues by capture immunoassays

Authors: *W. BROWN, T. ADAYEV, R. KASCsAK, R. KASCsAK, C. DOBKIN, S. NOLIN, G. LAFAUCI;
Dept Human Genet., Inst. Basic Res., Staten Island, NY

Abstract: The Fragile X syndrome is due to mutations of the FMR1 gene that result in the absence of fragile X mental retardation protein (FMRP). We have developed a rapid, highly sensitive method for quantifying FMRP from dried blood spots and lymphocytes. This assay uses two new antibodies mAb 6B8 (Biolegend) and R477, a bacterially expressed abbreviated FMRP standard, and a Luminex platform to quantify FMRP. The assay readily distinguishes between samples from males with fragile X full mutations and samples from normal males. It also differentiates mosaic from non-mosaic full-mutation male samples. We have employed the assay to screen 2000 newborn dried blood spots (DBS) and present their distribution. We also applied the assay in a retrospective study of 76 newborn DBS that had been stored for an extended period and included full mutation males as well as normal individuals. We were able to correctly identify all 5 known male fragile X positive cases among samples stored up to 47 months. Using mAb 5C2 (Biolegend) and R477, we have also developed a similar immunoassay for the quantification of Fmrp in mouse tissues. This assay was used to quantify Fmrp in brainstem, cerebellum, hippocampus, and cortex strains of mouse (C57 BL and FVB) in seven and ten week-old animals, showing developmental variation. The latter assay will allow studying the developmental variation of Fmrp expression in different organs.

Title: A new proteomic profiling approach reveals dysregulation in ASD proteomes

Authors: *S. ARYAL*1,2, A. BHATTACHARYYA2, H. BOWLING2, G. ZHANG1, P. SMITH3, K. KIRSHENBAUM1, M. CHAO1, T. NEUBERT1, C. VOGEL4, E. KLANN3; 

Abstract: Autism spectrum disorder (ASD) affects 1 in 68 children in the U.S. One of the hypotheses for a common molecular mechanism underlying certain ASDs is dysregulated protein synthesis that most likely arises from altered translational control. Mouse models that exhibit dysregulated translation, such as elf4E transgenic mice (elf4E Tg) and fragile X syndrome (FXS) model mice, display characteristic deficits in synaptic physiology and behaviors reminiscent of ASD. Therefore, identification and quantitation of the proteins that are synthesized and expressed aberrantly in the intact circuits of these ASD model mice could yield important clues in the pathophysiology of ASD. To accomplish this, we utilized an in-house developed combinatorial method of proteomic profiling that we term BONLAC (BONCAT-SILAC), which combines two established protein detection and profiling techniques. Using the technique, we were able to assess de novo protein synthesis and altered protein expression in hippocampal slices of elf4E Tg and FXS mice as compared to their wild-type littermates. Using rank-order statistics based analysis, we measured over 500 consistently dysregulated de novo synthesized proteins in FXS mice and over 300 such proteins in elf4E Tg mice, respectively. We also performed Gene Ontology and functional clustering analyses, which revealed enrichment of synaptic, cytoskeletal, and metabolic proteins in our results across the two ASD models. We
validated several of the candidate proteins using Western blots. We also performed bioinformatics analysis of the mRNA sequences of the newly synthesized proteins to explore any commonalities in sequence features that could serve as potential sites of convergent cis-regulation. Our results suggest that mRNA sequences of the proteins that are upregulated and downregulated in the eIF4E Tg and FXS mice have different cis-regulatory signatures in their untranslated regions. Our findings reveal systems level insights on the altered translational landscape in intact circuits of two mouse models of ASD.


Poster

491. Fragile X Syndrome

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 491.23/E41

Topic: C.06. Developmental Disorders

Support: FRM

INSERM

Fondation Jérome Lejeune

Title: Defects in tactile stimulus evoked responses of layer 2/3 pyramidal neurons in the Fmr1-/-y mouse model of Fragile X Syndrome

Authors: *A. A. FRICK, A. BHASKARAN, K. LE CORF, M. GINGER, G. BONY; INSERM U862, Neurocentre Magendie, Bordeaux, France

Abstract: Fragile X Syndrome (FXS) is the most common form of inherited mental retardation syndrome and a frequent cause of autism spectrum disorders (ASD). Defects in sensory information processing are a common feature of both FXS and ASD, but their underlying mechanisms are poorly understood. To understand the cellular and network basis of this phenomenon, we measured the sensory stimulus evoked properties of pyramidal neurons of layer (L) 2/3 of the primary somatosensory (S1) cortex of Fmr1-/-y mice and their wildtype WT) littermates. Whole cell patch-clamp recordings were performed in the hindlimb area of S1 in anesthetized animals (P24-31) and sensory responses evoked using stimulation of the contralateral hindpaw. A companion poster (Bony et al., SFN 2015) describes the intrinsic
properties of these neurons and their sensory receptive fields under normal physiological conditions. In the Fmr1−/y mouse, sensory stimulation evoked postsynaptic potentials of similar amplitude (c.f. WT mice; ~9 mV). However, we found an increased onset latency and half-amplitude duration (P < 0.05). Moreover, our preliminary also data suggest an increase in the hindpaw stimulus-evoked action potential activity in the Fmr1−/y mice. In addition, these neurons showed a greater propensity for responding to forepaw stimulation, suggesting an alteration of the receptive field properties. We also found a change in certain intrinsic properties of L2/3 pyramidal neurons. In contrast, we observed no effect of genotype on the spontaneous activity of these neurons. Lastly we present evidence suggesting that changes in their connectivity contribute, at least in part, to the exaggerated response to sensory stimuli observed in Fmr1−/y mouse.

**Disclosures:** A.A. Frick: None. A. Bhaskaran: None. K. Le Corf: None. M. Ginger: None. G. Bony: None.

**Poster**

**491. Fragile X Syndrome**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

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**Topic:** C.06. Developmental Disorders

**Support:** NIH Grant R01MH091186

Pew Charitable Trust Grant 2009-000359-016

University of Michigan Protein Folding Disease Initiative Grant

**Title:** Dysregulated Dscam levels act through Abelson tyrosine kinase to enlarge presynaptic arbors

**Authors:** *G. R. STERNE*, J. KIM, B. YE;
Life Sci. Inst., Univ. of Michigan, Ann Arbor, MI

**Abstract:** Increased expression of Down syndrome cell adhesion molecule (Dscam) is implicated in the pathogenesis of several brain disorders, including Down syndrome, intractable epilepsy, and possibly Fragile X syndrome (FXS). Though targeting the overabundance of Dscam or its downstream signaling might yield novel therapeutics for these disorders, little is known about Dscam’s downstream signaling. Here, we show that the cellular defects caused by dysregulated Dscam levels can be ameliorated by genetic and pharmacological inhibition of
Abelson kinase (Abl) both in an experimental model and a *Drosophila* model of fragile X syndrome. This study establishes Abl as a downstream effector of Dscam function and offers Abl as a potential therapeutic target for treating brain disorders associated with dysregulated Dscam expression.

**Disclosures:**  
**G.R. Sterne:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent application number: PCT/US2014/072083.  
**J. Kim:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent application number: PCT/US2014/072083.  
**B. Ye:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent application number: PCT/US2014/072083.

**Poster 491**  
**Fragile X Syndrome**  
**Location:** Hall A  
**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM  
**Program#/Poster#:** 491.25/E43  
**Topic:** C.06. Developmental Disorders  
**Support:** NIH/NIMH R01 MH092877-01  
**Title:** The actin-depolymerizing factor, coflin, plays a critical role in the dendritic spine abnormalities associated with fragile x syndrome  
**Authors:** *A. PYRONNEAU*¹, R. S. ZUKIN²; ¹Neurosci., ²Albert Einstein Col. of Med., Bronx, NY  
**Abstract:** Fragile X Syndrome (FXS) is the most common form of inherited intellectual disabilities and autism. The primary cause is an unstable CGG trinucleotide repeat expansion leading to hypermethylation and transcriptional silencing of the *FMR1* gene. The Fragile X Mental Retardation Protein (FMRP), the *FMR1* gene product, is an mRNA binding protein that represses >1000 mRNA targets. Loss of FMRP causes unchecked translation of neuronal proteins normally regulated by FMRP. The neuroanatomical hallmark of FXS is an overabundance of long, thin dendritic spines. However, the molecular mechanisms linking loss of FMRP to aberrant spine morphology remain unclear. Rac1 is a member of the Rho family of small GTPases, which regulates the actin cytoskeleton. Rac1 promotes phosphorylation/inactivation of the actin depolymerizing factor coflin, enhanced actin polymerization, and spine remodeling. The underlying hypothesis of my research is that loss of
FMRF causes enhanced Rac1 activity, inactivation of cofilin and an overabundance of immature spines. Here we show that the actin depolymerizing factor cofilin, which is a major contributor of dendritic spine structure, is dysregulated in FXS and contributes to these spine abnormalities. We found that cofilin phosphorylation is elevated in Fmr1 KO mouse somatosensory cortex at an age of substantial synaptogenesis (1 week) but not at later ages (4 weeks). In addition, consistent with aberrant cofilin signaling we show (1) the Rho GTPase, Rac1, exhibits elevated activity, (2) PAK1 (a Rac1 effector) exhibits elevated activity, (3) phosphorylation and inactivation of Slingshot and phosphorylation/activation of Lim kinase (two key downstream targets of PAK1 and upstream regulators of cofilin) are elevated, (4) abundance of profilin 2 (an actin polymerizing factor downstream of Rac1) is elevated and (5) F/G actin ratio, a functional readout of cofilin, is elevated in the somatosensory cortex of 1 week old Fragile X mice. Furthermore inhibition of PAK with a small molecule inhibitor completely restores cofilin signaling in Fragile X mice. Moreover viral delivery of a constitutively actin cofilin mutant (cofilin S3A) in the somatosensory cortex of young Fmr1 KO mice rescued the immature dendritic spine phenotype indicating a causal relation between elevated cofilin phosphorylation and aberrant dendritic spine morphology in Fragile X. These findings are consistent with a model whereby elevated Rac1/cofilin signaling plays an important role in the dendritic spine defects in Fragile X.

Disclosures: A. Pyronneau: None. R.S. Zukin: None.

Poster

492. Epilepsy Network and Synaptic Mechanisms

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 492.01/E44

Topic: C.07. Epilepsy

Support: NS34774

Title: Thalamic hyperexcitability in the Scn8a model of absence epilepsy

Authors: *C. D. MAKINSON1, J. SOROKIN1, C. A. CHRISTIAN2, J. R. HUGUENARD2; 1Neur., 2Stanford Univ., Palo Alto, CA

Abstract: Absence epilepsy is a CNS disorder characterized by loss of consciousness and spontaneous generalized spike-and-wave discharges (SWDs). The underlying pathophysiology of SWDs is thought to involve dysfunction of the thalamo-cortical loop, which is comprised of reciprocal glutamatergic corticothalamic (CT) and thalamocortical (TC) connections and GABAergic inhibition from the thalamic reticular nucleus (nRT) onto TC neurons. In fact,
deficits in the synaptic and intrinsic excitability of these cell types have been observed in models of absence epilepsy. Recently, absence seizures have been observed in humans and in mice carrying mutations in the voltage-gated sodium channel (VGSC) gene Scn8a, encoding the protein Nav1.6. Nav1.6 strongly modulates neuronal excitability and is important for initiating and propagating action potentials. Given the established importance of thalamic networks to the expression of absence seizures, we sought to evaluate the possibility that loss of Scn8a leads to hyperexcitability of cortical or thalamic networks contributing to absence seizures in these mice. To this end, we performed extracellular multielectrode and whole-cell electrophysiological recordings of critical cell types and regions within the thalamo-cortical loop of Scn8a deficient animals. We found that isolated thalamic networks from Scn8a-deficient mice are in fact susceptible to the generation of spontaneous epileptiform oscillations. Additionally, electrically-evoked oscillations in Scn8a-deficient animals persisted longer (wild-type 1.8 ± 0.7 sec vs. Scn8a+/- 6.9 ± 1.5 sec) and involved a greater number of burst discharges (wild-type 12.1 ± 4.5 vs. Scn8a+/- 64.6 ± 17.7) than wild-type littermate controls. Furthermore, we observed reduced tonic and burst firing behavior in nRT neurons, which are the sole inhibitory GABAergic cell type within this thalamic network, but not in glutamatergic TC neurons, from Scn8a mutant animals compared to wild-type controls. These results document a specific dysfunction in thalamic inhibition, which may contribute to the observed hyper-oscillatory activity of the thalamocortical circuit. Additionally, these findings reveal the importance of Scn8a in shaping thalamic activity and point to a novel mechanism of SWD generation.


Poster

492. Epilepsy Network and Synaptic Mechanisms

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 492.02/E45

Topic: C.07. Epilepsy

Support: AES Seed Grant

Title: Excitability of CA3 hippocampal neurons in a mouse model of Dravet syndrome

Authors: *H. B. FERNANDES1, J. A. KEARNEY2, A. L. GEORGE, Jr.2, A. CONTRACTOR1;
1Physiol., 2Pharmacol., Northwestern Univ., Chicago, IL
Abstract: Dravet syndrome is an epileptic disorder that results from mutations that produce haploinsufficiency of the Scn1a gene that codes for the Na\textsubscript{V}1.1 sodium channel subunit. Mutations in this gene produce a disorder characterized by generalized seizures with a febrile component starting in infancy, and with a parallel cognitive delay independent of seizure activity. While a reduction in sodium channel expression may be expected to produce an overall reduction in network excitability, a number of studies have demonstrated that Na\textsubscript{V}1.1 is selectively expressed in GABAergic interneurons in the forebrain. Hence haploinsufficiency of Scn1a paradoxically results in enhanced network excitability, attributed to a reduction in inhibition. Recent evidence using isolated hippocampal pyramidal neurons from a knock-in mouse model of Dravet syndrome suggests that parallel changes in other sodium channel subtypes may occur concomitantly to changes in Na\textsubscript{V}1.1 expression, however the precise changes in neuronal excitability in different hippocampal regions in this mouse model are still unknown. To determine how haploinsufficiency of Scn1a affects excitability of neurons during a critical period (P21-P25) in the hippocampus, we used single cell electrophysiological recording of hippocampal neurons in acute slices from Dravet mice and wild-type (WT) littermates. This approach has several advantages over studies in isolated cells. Inhibitory influences on cell excitability remain intact, and a more precise identification of neuronal subtype can be made based on neuroanatomical region and cellular morphology. Previous studies in Dravet mice have largely focused on the CA1 region of the hippocampus but nothing is known about possible alterations in intrinsic excitability of neurons within the CA3 region. This is a critical knowledge gap, as the CA3 auto-associational network is an ideal locus for seizure generation and propagation. We used whole-cell patch clamp in both voltage and current clamp modes to examine cellular excitability and ion channel current amplitudes in both pyramidal cells and interneurons within the CA3 region of hippocampal slices. Comparative measurements were made of intrinsic properties, neuronal excitability, and synaptic properties between Dravet mice and littermate controls. These data will provide insight into the cellular and synaptic changes that occur in the CA3 region of the Dravet syndrome mouse model.


Poster

492. Epilepsy Network and Synaptic Mechanisms

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 492.03/E46

Topic: C.07. Epilepsy
Support: Excellence Cluster ‘BrainLinks-BrainTools’ (DFG grant EXC1086)
Research Commission, Medical Faculty Freiburg

Title: Altered connectivity of the hippocampal CA2 region in temporal lobe epilepsy

Authors: *U. HAUSSLER¹, J. SULGER¹, K. RINAS¹, A. KILIAS², C. A. HAAS¹,⁴
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Abstract: In temporal lobe epilepsy (TLE) seizures are often accompanied by structural changes in the hippocampus such as cell loss in the CA3 and CA1 region and hilus, reactive gliosis and granule cell dispersion. In addition, sprouting of mossy fibers (MF) leads to aberrant recurrent connectivity in the dentate gyrus. In contrast, the CA2 region seems rather resistant to the pathological changes. Nonetheless, the structure and connectivity as well as the functional role of CA2 in TLE have not been thoroughly investigated yet. Only recently, MF input to CA2 has been shown in the healthy hippocampus (Kohara et al., 2013, Nature Neuroscience; Hitti and Siegelbaum, 2014, Nature), based on a molecular definition of CA2 which contrasts the traditional view which assigned CA2 as the region adjacent to CA3 but without MF input. We used a mouse model for TLE to investigate the integration of CA2 in the epileptic hippocampal network and asked (i) whether the MF input to CA2 is altered, (ii) where CA2 output projections target to, since CA1 suffers from cell loss and (iii) whether CA2 contributes to epileptic activity. To this end we injected kainate (KA) into the septal hippocampus of C57Bl/6 and transgenic Thy1-eGFP mice (M-line, Feng et al., 2008, Neuron), which express eGFP mainly in dentate granule cells and MF, and implanted intrahippocampal electrodes for in vivo recordings. Subsequently, we performed immunocytochemistry with CA2-specific antibodies (regulator of G-protein signaling 14 and Purkinje-cell protein 4) and synaptoporin to detect MF synapses. In addition, we injected a fluorescence-tagged adeno-associated virus into CA2 to trace its projections. We show that CA2 pyramidal cells survive after KA injection but are dispersed resulting in an elongated CA2 region. Concomitantly, MF undergo major reorganization processes and sprout into the pyramidal cell layer of CA2 where eGFP+synaptoporin double-positive MF boutons surround pyramidal cell somata, introducing somatic in addition to dendritic excitation. On the postsynaptic side, CA2 projects to CA1 in the temporal hippocampus and to the contralateral hippocampus indicating its network integration. Epileptic activity in CA2 occurs only with a delay relative to the dentate gyrus indicating that CA2 rather transfers than generates epileptic activity, yet, the development of fast ripples in CA2 during epileptogenesis points towards a functional impact of the altered synaptic integration of CA2. Supported as part of the Excellence Cluster ‘BrainLinks-BrainTools’ (DFG grant EXC1086); Research Commission, Medical Faculty Freiburg.

Poster

492. Epilepsy Network and Synaptic Mechanisms

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 492.04/E47

Topic: C.07. Epilepsy

Support: DFG grant EXC1086

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BMBF grant FKZ 1GQ0830

ERA-Net NEURON II CIPRESS

Schram Foundation

Title: Structural and functional plasticity of entorhinal input contributes to an epileptic hippocampal circuitry


Abstract: Dentate granule cells (DGCs) receive their major excitatory input from the entorhinal cortex through the perforant path. Although several studies emphasized a role for the entorhinal cortex in epilepsy, it remains uncertain whether its synaptic connections with DGCs are altered. We therefore asked if synaptic inputs from the medial entorhinal cortex (MEC) via the medial perforant path (MPP) are morphologically and functionally changed in chronic epilepsy. To address this question, we first induced mesial temporal lobe epilepsy in adult C57Bl/6 or transgenic Thy1-EGFP mice by injecting kainate into one side of the hippocampus. Saline injections served as controls. Subsequently, we traced the MPP by stereotactic infusion of an anterograde neuronal tracer (biotinylated dextran amine) into the MEC 14 days later. After a survival period of 21 days, brain sections were immunohistochemically processed for identification of MPP fibers, localization of their presynaptic terminals and postsynaptic
partners, using antibodies against biotin, vGLUT-1 and PSD-95 respectively. Quantitative analysis was performed by applying Imaris-based 3D reconstruction. Furthermore, we investigated the ultrastructure of MPP synapses with electron microscopy. Functional properties were inferred in acute slice preparations, by combining whole-cell patch-clamp recordings of individual DGCs and simultaneous extracellular stimulation within the MPP termination zone. We show that under epileptic conditions the MPP is preserved on both the mesoscopic and the synaptic level, despite severe hippocampal sclerosis and granule cell dispersion. Remarkably, MPP synapses exhibited ultrastructural changes such as enlargement of presynaptic boutons and postsynaptic spines, increase in size and complexity of postsynaptic densities, and number of putative contacts. These structural changes are similar to the ones observed upon induction of long-term synaptic potentiation. Moreover, spine densities increased at dendritic segments within the MPP termination zone, and immunohistochemical staining for PSD-95 and vGLUT-1 suggested that newly generated spines are functionally mature and receive glutamatergic input. Finally, whole-cell recordings of DGCs indicated an increase in maximal amplitude of evoked excitatory postsynaptic potentials in the epileptic hippocampus. In conclusion, our findings suggest that structural and functional changes of MPP synapses contribute to an epileptic hippocampal circuitry. Supported by the DFG within the Cluster of Excellence “BrainLinks-BrainTools” (DFG grant EXC1086), FOR2143 (MB) and the Schram Foundation (MB).


Poster
492. Epilepsy Network and Synaptic Mechanisms

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

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Topic: C.07. Epilepsy

Support: German Federal Ministry of Education and Research FKZ 01GQ0420

    German Federal Ministry of Education and Research 01GQ0830

    German Science Foundation within the Cluster of Excellence BrainLinks-BrainTools (EXC 1086)

    European Union/European Regional Development Fund (ERDF) and INTERREG IV Upper Rhine (Project TIGER)
Title: Theta oscillation impaired along the septo-temporal axis of the epileptic hippocampal formation

Authors: *A. KILIAS*1,2,3, U. HÄUSSLER4, K. HEINING1,2,3, U. P. FRORIEP5, A. KUMAR6, C. A. HAAS4,3, U. EGERT1,2;  
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Abstract: Mesio-temporal lobe epilepsy (MTLE) manifests in the hippocampal formation as recurrent epileptic activity (EA) and histopathological changes. While the anatomical changes are persistent, EA is intersected by periods of putatively normal brain activity. These EA-free periods comprise network oscillations that are well studied in the healthy hippocampus. We investigate these rhythms in the epileptic brain and search for altered network properties that render the hippocampal formation susceptible to seizures. We investigate these network oscillations in the intrahippocampal kainate mouse model of MTLE, in which a unilateral injection of kainate into the septal dentate gyrus (DG) induces histopathological changes resembling human MTLE. The severity of these changes decreases towards the temporal pole of the hippocampus. Recently we showed that the power of EA in the DG is unequally distributed along the gradually changed septo-temporal axis (Häussler et al., 2012) but it remains an open question whether theta band power and frequency also show position-dependent changes. We further found that the coupling of theta rhythms between medial entorhinal cortex (MEC) and the sclerotic septal DG is phase-shifted in epileptic animals (Froriep et al., 2012), but it was unknown whether this shift persists along the septo-temporal axis and whether it is accompanied by corresponding changes in neuronal firing. To address these questions, we implanted wire electrodes and custom-made multi-site silicon probes at several positions of the hippocampal formation (HF) and simultaneously recorded local field potentials (LFPs) and multi-unit activity (MUA) in freely behaving mice. We observed theta oscillations at all tested septo-temporal locations in the DG and in the MEC of epileptic animals. Its mean frequency, however, was reduced throughout the extent of the DG and in the MEC of epileptic compared to healthy animals. In contrast, theta band power increased in the MEC but decreased in the septal sclerotic DG. In line with prominent theta oscillations throughout the HF, neurons in all substructures fired phase locked to the rhythm. Neurons of the weakly sclerotic temporal DG as well as of the MEC and Parasubiculum showed a preserved theta phase distribution of firing compared to those obtained from healthy mice. Even though theta oscillations in epileptic animals are slower and power is unequally distributed across the HF neurons still fire phase coupled to the rhythm. Supported by the BMBF (FKZ 01G00420 and 01GQ0830) and by the DFG within the Cluster of Excellence BrainLinks-BrainTools (EXC 1086). Cofinanced by the EU/ERDF and INTERREG IV Upper Rhine (Project TIGER).
Title: Two-photon imaging reveals the population dynamics of spatiotemporally compartmentalized ictal networks

Authors: *M. WENZEL*, J. P. HAMM, D. S. PETERKA, R. YUSTE; Columbia Univ. / Biol. Sci., New York, NY

Abstract: Recent advances in understanding epileptic population dynamics have challenged the classical view of epilepsy as a condition of stereotyped ictal events. Several studies have revealed far more heterogeneous epileptic network activity patterns at the microscale than previously appreciable at a macroscopic level. Yet, studies on ictal networks with fine spatiotemporal resolution have remained sparse, especially in the intact brain. It remains a question of great conceptual and therapeutic interest if reliable patterns of neural recruitment to ictal activity exist at the level of individual assemblies or cells. As for the neural network as a whole, progress towards greater complexity has also been seen regarding the role of neuronal subtypes in epilepsy. While numerous studies indicate that the failure of local inhibition is causally related to ictal expansion and that the activation of interneurons might help suspending seizures, others have observed interneuronal involvement in the induction of epileptic discharges. We combine LFP recordings with 30Hz resonant two-photon calcium imaging in a mouse model of acute pharmacological seizures (4-AP) allowing us to study neural population dynamics during ictogenesis and epileptic spread, with single cell precision. We observe strikingly differential intra-focal and penumbral network activity dynamics with saltatory ictal progression within the epileptic focus versus a smooth invasion of the ictal penumbra. We show that the epileptic network displays partially reliable spatio-temporo-progressive neural recruitment in both cortical compartments, even at the single cell level. This reliability is found despite great temporal network variability consistent with a stretchable neural mesh where ictal
progression may vary in time but cannot betray current neural connectivity. Finally, we perform population imaging of parvalbumin positive interneurons (PVs) and confirm that they form integral part of the inhibitory restraint, yet also observe unexpectedly non-participant PVs during maximal network excitation and provide insight into how this neural subtype could in fact also support the formation of a critical hyperexcitatory mass. Supported by NIH (R01EY011787) and DFG (We 5517/1-1).


Poster

492. Epilepsy Network and Synaptic Mechanisms

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 492.07/F2

Topic: C.07. Epilepsy

Support: NIH/DO Grant

HHMI-CURE Medical Research Fellowship

Title: Understanding the role of interneurons in seizure initiation, propagation, and termination using an optogenetic mouse model of seizures

Authors: *S. KHOSHKHOO1, V. SOHAL2;
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Abstract: Epilepsy is a disorder of brain microcircuits that arises from aberrant neuronal synchronization. Traditionally, this has been attributed to excessive levels of excitation that is able to overcome inhibitory regulation by GABAergic interneurons within abnormal neuronal circuits. However, recent studies have raised the possibility that selective optogenetic activation of interneurons, can not only terminate, but also, under the right conditions, initiate, seizures in the mouse hippocampus. Similarly, within [in vitro] models of seizures, optogenetic inhibition of PV interneurons can reduce the frequency of spontaneous epileptiform discharges. These findings inspired us to further investigate the role of parvalbumin (PV), somatostatin (SOM), and vasoactive intestinal peptide (VIP) interneurons during seizures. To facilitate temporally specific, on-demand seizure initiation, we developed an [in vivo] optogenetic kindling model of ictogenesis by focally expressing ChR2 within excitatory neurons in the mouse neocortex. We simultaneously carried out cell type-specific bulk calcium imaging in the contralateral cortex using Cre-dependent expression of the genetically encoded calcium indicator, GCaMP6f, within
PV, SOM, or VIP interneurons, or excitatory neurons. We find distinct, cell-type specific temporal patterns of activity relative to seizure onset or termination. In particular, our results suggest that specific classes of GABAergic neurons may play important roles in the initiation and spread of seizures, in addition to seizure termination. In the future, we hope to take advantage of our novel experimental setup to further elucidate the functional significance of different interneurons in seizures using optogenetic manipulation.

Disclosures: S. Khoshkhoo: None. V. Sohal: None.

Poster

492. Epilepsy Network and Synaptic Mechanisms

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 492.08/F3

Topic: C.07. Epilepsy

Title: Functional impact of minimal KCC2 in the reticular thalamus on regulation of thalamic oscillations

Authors: *P. KLEIN, M. E. HARPER, P. A. DAVOUDIAN, M. P. BEENHAKKER; Univ. of Virginia, Charlottesville, VA

Abstract: Absence seizures, which fall within the larger spectrum of epileptic disorders, are defined by recurrent 3 Hz spike-wave cortical discharges and accompanying loss of consciousness. These seizures are characterized by increased activity in the thalamus, which is produced by an oscillatory circuit between thalamocortical and reticular thalamic neurons. This typically produces bursts of activity during sleep, yet a prevalence of evidence indicates that absence seizures occur when the mechanisms which typically desynchronize activity within the thalamic circuit become disrupted, and activity becomes highly synchronized and generalizes to other regions of the brain. GABAergic signaling within the reticular thalamic nucleus is believed to prevent hypersynchronous firing, yet the exact mechanism by which this occurs is still an active subject of debate. Indeed, studies in the last few years have proposed an inhibitory, an undetectable or even an excitatory role for GABAergic signaling within the reticular thalamic nucleus. While the combined evidence is most supportive of an excitatory role, the mechanisms by which such signaling between reticular thalamic neurons constrains network excitability are unknown. The reversal potential for GABAergic currents is largely determined by intracellular chloride concentrations, which in turn are established by the ion transporters NKCC1 and KCC2. Developmental regulation of NKCC1 and KCC2 in the thalamus has not previously been examined. Using immunohistochemistry, we have found that throughout development, reticular
thalamic neurons in rats largely lack expression of KCC2, consistent with previous findings in
adult mice (Sun et al., 2012). This reduced expression of KCC2 in reticular thalamic neurons
indicates that intracellular chloride levels are elevated and suggests that seizure suppressing
connectivity between reticular thalamic neurons is the result of excitatory GABAergic signaling.
Indeed, through calcium imaging of population level activity within the thalamus, we have found
that addition of the NKCC1 antagonist bumetanide is capable of increasing both amplitude and
synchrony of activity between reticular thalamic neurons. A more complete and accurate
understanding of the baseline and seizure-generating states of the thalamic oscillatory circuit will
provide opportunities for both improved knowledge of the role the thalamus plays in epilepsy, as
well as insights for improved targeting and development of therapies.

None.

Poster

492. Epilepsy Network and Synaptic Mechanisms

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Program/#Poster#: 492.09/F4

Topic: C.07. Epilepsy

Support: 2015AA020512

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Title: Optogenetic dissection of ictal propagation of temporal lobe epilepsy in hippocampal-
entorhinal cortex circuitry

Authors: *Y. LU, C. ZHONG, Y. ZOU, L. WANG;
Shenzhen Inst. of Advanced Technologies, Chinese Acad. of Sci., Guandong, China

Abstract: Temporal lobe epilepsy (TLE) is one of the most common drug-resistant forms of
epilepsy in adults and usually originates in the hippocampal formations. However, network
mechanisms that support seizure spreading and the exact directions of ictal propagation remain
largely unknown. To address the circuit-level mechanisms controlling seizure activity, we
developed novel neural probes for drug delivery, as well as multi-site optical stimulation and
electrophysiological recordings in the hippocampal-entorhinal cortex structures \textit{in vivo}. We induced TLE seizures by direct micro-injection of Kainic Acid (KA) into the dorsal hippocampus of VGAT-ChR2 transgenic mice, and performed multi-channel recordings from multiple brain regions. Based on the multi-region electrical recordings, we found significantly increased synchronizations between the recorded regions during ictal seizures. We calculated the temporal relationship between neural activities recorded in these brain regions, and identified a dominate propagating direction of ictal discharges. We also demonstrate that activating GABAergic interneurons (INs) in the upper stream of the epileptic circuitry can significantly inhibit the spreading of ictal seizures and largely rescue the behavioral deficits in KA-treated animals. These findings may have implications for existing and future therapeutic treatments of TLE.

**Disclosures:** Y. Lu: None. C. Zhong: None. Y. Zou: None. L. Wang: None.

**Poster**

492. Epilepsy Network and Synaptic Mechanisms

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program/#Poster#:** 492.10/F5

**Topic:** C.07. Epilepsy

**Support:** Spanish Ministerio de Economía y Competitividad Grant (BFU2012-37156-C03-01) Spanish Ministerio de Educación, Cultura y Deporte PhD fellowship (FPU12/03776)

**Title:** Heterogeneous pyramidal cell dynamics of high-frequency oscillations in an experimental model of temporal lobe epilepsy

**Authors:** *M. VALERO*\(^1\), J. AGUILAR\(^2\), E. CID\(^1\), L. MENÉNDEZ DE LA PRIDA\(^1\);
\(^1\)Functional and Systems Neurobio., CSIC, Madrid, Spain; \(^2\)Hosp. Nacional de Parapléjicos de Toledo, Toledo, Spain

**Abstract:** Transient high-frequency oscillations (HFOs; 100-600 Hz) have been implicated in both physiological and pathological processes in human temporal lobe epilepsy (TLE). Two major pathological forms of HFOs have been described in the epileptic hippocampus: those associated to interictal spikes (IID-HFOs), and ripple-like HFOs that invade the fast ripple band (FR-HFOs). In this study we investigate the cellular basis of these two forms of HFOs in TLE rats with a combination of multite silo probe and intracellular sharp recordings and anatomical labeling. Under urethane anaesthesia, we found IID-HFOs and FR-HFOs of similar
features than those recorded in freely moving TLE rats. FR-HFOs events were typically recorded at the CA1 cell layer in association with a sharp-wave (SPW) of several hundred microvolts at the stratum radiatum (10/14 rats), similar to physiological SPW-ripples. The remaining rats (4/14) showed SPWs at the radiatum with disorganized multi-unit firing at the cell layer. A half of the animals (714) displayed IID-HFOs at the CA1 cell layer with a large spike at the radiatum in the millivolt scale. IID-HFOs and FR-HFOs coexisted in 4/7 rats. Intracellularly, FR-HFOs were typically associated with the influence of predominant depolarizing potentials (14/20 cells from 10 rats), most of which exhibited an up-regulated bursting phenotype (13/20 cells). In contrast, IID-HFOs were typically associated with an initial typically supratheshold depolarization followed by a large hyperpolarizing potential that correlated with decreased multi-unit activity (n=11 cells from 7 rats). We propose that different cellular processes underlie pathological forms of HFOs in the TLE hippocampus.

Disclosures: M. Valero: None. J. Aguilar: None. E. Cid: None. L. Menéndez de la Prida: None.

Poster

492. Epilepsy Network and Synaptic Mechanisms

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Topic: C.07. Epilepsy

Support: Hellman Foundation

Epilepsy Foundation

NRSA 1F32MH096526-01A1

Walter F. Heiligenberg Professorship

Title: Some high frequency oscillations are more normal than others: pHFOs and ripples in chronic epilepsy

Authors: *L. A. EWEll1, K. B. FISCHER1, S. LEUTGEB1,2, J. K. LEUTGEB1; 1Ctr. for Neural Circuits and Behavior, Div. of Biol. Sci., UC-San Diego, La Jolla, CA; 2Kavli Inst. for Brain and Mind, San Diego, CA

Abstract: Virtually all depth-recording studies from humans are performed in epilepsy patients who are candidates for surgical intervention. Human recording studies have provided a
framework for understanding the network dynamics that underlie human cognition, however, there always remains one caveat: the neural networks being studied are pathological and it is unclear to what extent normal memory mechanisms persist. In healthy hippocampal networks transient 150 - 250 Hz oscillations called sharp wave ripples (SWRs) reflect reactivations of neural ensemble activity that represent a past experience, and are thought to be necessary for memory consolidation. In epilepsy networks, pathological high frequency oscillations (pHFOs) emerge, and are similar to SWR in duration and sometimes frequency. It is currently unknown whether epilepsy networks still participate in normal SWR. To study this question, rats with chronic epilepsy (n = 4) and control rats (n = 4) were implanted with an electrode assembly that targeted the hippocampus. Rats were trained to forage in an open field while hippocampal local field potentials and CA1 single units were recorded. Foraging sessions were flanked with resting sessions so that sleep-related activity could be recorded. SWRs in control animals (n = 691, recorded during sleep and exploration) had an average frequency of 189 ± 0.7 Hz and were associated with a sharp wave voltage deflection that had an average amplitude of 308 ± 6.8 μV. When the same parameters were measured and plotted for HFOs recorded in chronically epileptic rats during sleep and exploration, three clusters emerged. One cluster was distinguished by a very large voltage deflection (an inter-ictal spike). HFOs in this cluster (n = 131) had an average frequency of 253.6 ± 1.9 Hz and were associated with inter-ictal spikes with an average amplitude of 795.1 ± 24.6 μV. Both the frequency and voltage deflection amplitude were significantly different from control (p < 0.001). Similarly the HFOs in the second cluster (n = 377) were clearly outside of the normal physiological range with an average frequency of 258.7 ± 1.8 Hz and inter-ictal spike amplitude of 411.8 ± 6.5 μV (p < 0.001). Interestingly the third cluster (n = 275) completely overlapped with the control distribution with an average frequency of 188.6 ± 1.1 Hz and a sharp wave voltage deflection of 296 ± 8.0 μV, suggesting that CA1 networks in epilepsy are capable of generating activity patterns that appear normal in terms of shape parameters. Future work will assess if these ‘normal’ ripples follow control properties in terms of brain-state dependence and single unit recruitment in sleep and behavior.


Poster

492. Epilepsy Network and Synaptic Mechanisms

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 492.12/F7

Topic: C.07. Epilepsy

Support: Epilepsy Foundation (J.Y)
Title: Dentate cannabinoid-sensitive interneurons develop selective strengthening of mutual synaptic inhibition in experimental epilepsy

Authors: *V. SANTHAKUMAR, A. PRODDUTUR, B. SWIETEK, J. YU; Pharmacology, Physiol. & Neurosci., New Jersey Med. School, Rutgers, Newark, NJ

Abstract: Altered inhibition is a salient feature of hippocampal network reorganization in epilepsy. In the dentate gyrus, accommodating interneurons (AC-INs) including hilar interneurons with inner-molecular layer or total molecular layer axonal projections exhibit adapting firing and mediate cannabinoid receptor type 1 (CB₁R)-sensitive GABA release. Experimental epilepsy leads to changes in interneuronal CB₁R expression and reduction in CB₁R-sensitive GABAergic inputs to hippocampal and dentate projections neurons. Here we examined whether intrinsic physiology and inhibitory regulation of AC-INs is modified following pilocarpine-induced status epilepticus (SE). Single cell and paired recordings were obtained from interneurons in the granule cell layer-hilar border in hippocampal slices from male rats 5-10 days (post-SE) and > 40 days (epileptic, with spontaneous seizures) after pilocarpine induced SE and from age-matched, saline-injected controls. AC-INs showed no change in intrinsic active and passive properties in post-SE and epileptic rats. Unlike granule cells and fast-spiking basket cells, AC-INs showed no reduction in the frequency of spontaneous or miniature inhibitory postsynaptic currents (IPSCs) in post-SE and epileptic rats. In paired interneuronal recordings, the amplitude of unitary synaptic GABA currents between AC-INs doubled after SE (in pA, con: 19.4±2.6; post-SE: 38.7±5.5, p<0.05). Non-stationary noise analysis identified an increase in GABA receptor number at synapses between AC-INs after SE. However, baseline synaptic release and CB₁R antagonist-enhancement of release at AC-IN synapses were not different between control and post-SE rats. CB₁R agonist reduced the amplitude and suppressed a greater proportion of spontaneous IPSCs in AC-INs from post-SE and epileptic rats than in controls. These data demonstrate that dentate CB₁R-sensitive interneurons develop an early and persistent strengthening of mutual inhibition in epilepsy. The increase in frequency of CB₁R-sensitive sIPSCs in AC-INs was not associated with enhanced AC-IN excitability or changes in baseline endocannabinoid modulation suggesting that synapses between AC-INs may be preserved or increased after SE. Post-SE enhancement of inhibition between dentate AC-INs, which show facilitating short-term dynamics, could impact granule cell dendritic integration during normal activity and following dynamic increases in network excitability during seizures.

Disclosures: V. Santhakumar: None. A. Proddutur: None. B. Swietek: None. J. Yu: None.

Poster

492. Epilepsy Network and Synaptic Mechanisms
Title: Epileptic synapses trigger aberrant intrinsic plasticity in the dentate gyrus

Authors: *V. CREPEL, A. PERET, Y. MIRCHEVA, G. MARTI, J. ARTINIAN; INMED, INSERM & Aix-Marseille Univ. UMR901, Marseille Cedex09, France

Abstract: Patients with temporal lobe epilepsy (TLE) often display cognitive comorbidity in addition to recurrent seizures. However, the cellular mechanisms underlying the impairment of neuronal information processing remain poorly understood in TLE. Within the hippocampal formation, neuronal networks undergo a major reorganization, including the sprouting of mossy fibers in the dentate gyrus; they establish aberrant recurrent synapses between dentate granule cells and operate via postsynaptic kainate receptors. In this study, we tested the hypothesis that this aberrant local circuit is able to alter information processing of perforant path inputs which constitute the major excitatory afferent pathway from entorhinal cortex to dentate granule cells. Our present data, obtained in patch-clamp in hippocampal slices, revealed that stimulation of recurrent mossy fibers triggered a repetitive firing regime of dentate granule cells in TLE. When used as conditioning trains, it increased more than 3 fold their firing rate in response to perforant path inputs during at least 30 minutes, altering durably granule cells input-output operation without increasing the synaptic strength. The recurrent mossy fibers-evoked repetitive firing regime was due to an aberrant readout of synaptic inputs by kainate receptors and the augmentation of perforant path-evoked firing rate was dependent on Ca2+ influx but not on NMDA receptors. Furthermore, we demonstrated that this perforant path-evoked firing increase is due to the aberrant activity-dependent potentiation of the persistent sodium current altering intrinsic excitability of dentate granule cells. We propose that this aberrant activity-dependent intrinsic plasticity, which lastingly impairs the information processing of cortical inputs the in dentate gyrus, may participate in hippocampal-related cognitive deficits such as those reported in patients with TLE.

Disclosures: V. Crepel: None. A. Peret: None. Y. Mircheva: None. G. Marti: None. J. Artinian: None.
Poster

492. Epilepsy Network and Synaptic Mechanisms

Location: Hall A

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Program#/Poster#: 492.14/F9

Topic: C.07. Epilepsy

Support: F31NS080403-02
      RO1NS082046

Title: Epilepsy-induced dentate granule cell hyperactivation: progression and mechanisms in a mouse model of temporal lobe epilepsy

Authors: *C. G. DENGLER¹, C. YUE³, H. TAKANO¹², D. A. COULTER²³;
      ¹Neurosci., ²Univ. of Pennsylvania, Philadelphia, PA; ³Children's Hosp. of Philadelphia, Philadelphia, PA

Abstract: In addition to its cognitive functions, the dentate gyrus (DG) plays an important role in the regulation of pathological activation of the limbic system, functioning as a regulated gate, restricting relay of synchronous network activity associated with epilepsy. A critical component of this gating function is the reluctance of granule cells to activate and their concomitant sparse activation, both in vivo and in vitro. In both human and animal models of epilepsy, there are multiple epilepsy-associated disruptions occurring within dentate circuitry, including mossy fiber spouting, alterations in local inhibition, and aberrant neurogenesis. Given these circuit changes, we hypothesized that the network activation properties of dentate granule cells (DGCs) would be corrupted. We therefore conducted multicellular calcium imaging using fast sweptfield confocal microscopy to probe activation of DGCs in hippocampal slices prepared from mice in a pilocarpine status epilepticus (SE) model of epilepsy to understand how development of chronic epilepsy alters circuit function in the dentate gyrus during epileptogenesis. We assessed proportional activation of DGCs responding to perforant path stimulation and found that control slices showed relatively sparse activation with 20% DGCs responding. Slices prepared 1 week post-SE displayed a significant collapse in normal sparse firing and increased to 90%. At 2 weeks post-SE there transient, partial restoration to 40% activation, followed by a significant secondary increase in responses at 2-3 months (65%) and at >6 months post-SE (90%) as animals become chronically epileptic. We assessed both sIPSCs and mIPSCs during epileptogenesis and found reductions in normal inhibitory function correlating with timepoints during epileptogenesis when DGCs displayed hyperexcitability. We have also previously shown that chloride regulation is also disrupted during epileptogenesis. Using GABA-A receptor and...
KCC2 antagonists, we found that pharmacologically mimicking changes in inhibitory receptor function and chloride reversal potential were each individually sufficient to significantly disrupt the normal, sparse activation in DGCs increasing activation by 2-fold, and that disrupting both simultaneously produced an additive effect in increasing granule cell activation 4-fold. Given that the ensemble activation properties of DGCs are critical determinants in both hippocampal dependent cognitive function and control of aberrant excitation in the limbic system, erosion in these properties may play a critical role both in cognitive comorbidities and seizure propensity in epilepsy.

Disclosures:  C.G. Dengler: None.  C. Yue: None.  H. Takano: None.  D.A. Coulter: None.

Poster
492. Epilepsy Network and Synaptic Mechanisms
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Topic: C.07. Epilepsy
Support: T32 MH 17168-31
RO1 NS 082046
Title: A comparison of sparse activation in the dorsal and ventral dentate gyrus granule cells
Authors: *J. B. KAHN¹, H. TAKANO¹, D. A. COULTER¹²; ¹Univ. of Pennsylvania, Philadelphia, PA; ²Children's Hosp. of Philadelphia, Philadelphia, PA
Abstract: The hippocampus exhibits distinct anatomy and behavior along its septotemporal axis, including differing patterns of gene and receptor expression, behavioral impact, and anatomical projections. The hippocampus also expresses differences in disease: epilepsy induces greater circuitry changes in the ventral than the dorsal hippocampus. Pronounced differences in circuit activity between the dorsal and ventral hippocampal microcircuits CA1 and CA3 are well documented, but the dentate gyrus (DG) has yet to be investigated extensively in this context. We examined cellular activation of the DG’s principal cells, dentate granule cells (DGCs), which in a healthy brain have been shown to have sparse, selective activation characteristics. Previous in vivo tetrode work has shown that most of the DGCs do not fire in any spatial environment and remain functionally silent, while the small remaining population of DGCs fire in all environments. Our laboratory has similarly demonstrated this sparse activation, reporting only 5% of the DGC population activating in ventral hippocampal-entorhinal cortical slices. In this
study, we used two-photon multicellular calcium imaging in horizontal ventral hippocampal-entorhinal cortical slices and in coronal dorsal hippocampal slices. Slices were bulk loaded with the calcium indicator dye Fura-2 AM, which labels virtually the entire DGC population, visualized as POMC-Cre x Rosa tdTomato labeled DGCs. Maximal DGC population activation was determined by applying picrotoxin (50 uM), a GABA(A) receptor antagonist, to the bath at the end of the imaging session; cells that activated in the presence of picrotoxin were the denominator for the “percent of the activating population” calculation. A significantly larger proportion of the DGCs in dorsal slices activated in response to stimulation than our previously reported 5%; however, this result is a function of the stimulating electrode’s placement in the slice. Coronal hippocampal slices must be stimulated in the DG’s molecular layer, confounding perforant path afferent input with direct DGC dendritic stimulation. Positioning the stimulating electrode in a similar location in the ventral DG’s molecular layer, rather than the perforant path in the entorhinal cortex, removed any significant differences in proportional activation. Our findings support previous work suggesting that the DGCs act as a sparsely activating network and suggest that this sparsity is maintained along the septotemporal axis.


Poster

**492. Epilepsy Network and Synaptic Mechanisms**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 492.16/F11

**Topic:** C.07. Epilepsy

**Title:** T-type calcium channels facilitate neuronal hyper-excitability in epileptic subiculum neurons

**Authors:** M. K. PATEL, B. BARKER, *J. A. HOUNSHELL; Anesthesiol., Univ. of Virginia, Charlottesville, VA

**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. Recent studies have suggested a role for T-type Ca2+ channels in facilitating increases in neuronal activity associated with TLE. We sought to determine if T-type Ca2+ channels are involved in maintaining neuronal hyper-excitability in the subiculum of TLE rats. To establish a role for T-
type Ca2+ channels we used 1µM TTAP, a potent and selective blocker of T-type Ca2+channels. TLE in rats was induced by electrical stimulation of the hippocampus for 90 minutes to induce status epilepticus (SE). Rats having two or more spontaneous seizures per day by EEG, 3 months after SE were used in the study. Brain slices were prepared and membrane properties were recorded from subiculum pyramidal cells. Action potentials (APs) were evoked by a series of depolarizing current injection steps under whole-cell current clamp conditions. Subiculum pyramidal neurons from TLE rat brain slices demonstrated a higher AP firing frequency than control. Bath application of 1µM TTAP significantly reduced firing frequencies in both control and TLE neurons. 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 1µM significantly reduced the number of APs evoked in both control and TLE neurons. Rebound firing, firing believed to be largely driven by T-type Ca2+channels, is significantly decreased in control and TLE subiculum neurons in response to 1µM bath application of TTAP. In TLE, subiculum pyramidal neurons have a more hyperpolarized threshold (-50.4±0.6 mV) compared to controls (-45.7±0.3 mV). Bath application of 1µM TTAP however restores threshold properties (-44.6±1.7) in TLE subiculum neurons. These data suggest that T-type Ca2+ channels play an important role in controlling neuronal membrane excitability in subiculum neurons. Alterations in the activity of T-type Ca2+ channels could be important in facilitating neuronal hyper excitability in TLE.


Poster

492. Epilepsy Network and Synaptic Mechanisms

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

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Topic: C.07. Epilepsy

Support: NIH Grant NS058585

Title: Electrophysiological properties of age-defined dentate granule cells in a rodent model of temporal lobe epilepsy

Authors: *A. L. ALTHAUS1, S. J. MOORE2, H. ZHANG3, G. G. MURPHY2,4, J. M. PARENT3;
1Mol. and Behavioral Neurosci. Inst., 2Neurol., 4Physiol., 1Univ. of Michigan, Ann Arbor, MI
Abstract: Dysregulated hippocampal neurogenesis is a prominent feature of temporal lobe epilepsy (TLE). Anatomical data indicate that most dentate granule cells (DGCs) generated in response to an epileptic insult develop features that promote increased excitability, including ectopic location, persistent hilar basal dendrites (HBDs) and mossy fiber sprouting. However, some DGCs appear to integrate normally, and may promote reduced excitability. Using a retroviral (RV) GFP reporter to birthdate DGCs, our laboratory found that DGCs that were mature at status epilepticus (SE) are resistant to morphological abnormalities, while the majority of those born after SE display TLE-related pathology. This may suggest that post-SE generated DGCs promote pathological function while established DGCs retain normal function. To examine the relationship between DGC age and activity within an epileptic network, we used acutely prepared hippocampal slices to make whole-cell voltage clamp recordings from RV birth-dated DGCs born either neonatally, or during adulthood in an epileptic or intact animal. We found that, in TLE tissues, both adult-born and neonatal-born populations of DGCs appear to receive increased excitatory input compared with age-matched controls in intact tissues. Furthermore, adult-born DGCs that display aberrant morphology in TLE tissues appear to receive more excitatory input than their normotopic counterparts. In particular, ectopic DGCs receive the greatest amount of excitatory input and least amount of inhibitory input when compared to other age and morphology defined groups of DGCs. Taken together, these data suggest that aberrant adult-born DGCs may underlie pathophysiological network mechanisms that promote epileptogenesis.


Poster

492. Epilepsy Network and Synaptic Mechanisms

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Topic: C.07. Epilepsy

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Title: Altered GluA2 expression provides a mechanism for efficacy of AMPA receptor blockade in preventing acute post-seizure cellular changes in the developing hippocampus
Authors: *J. J. LIPPMAN BELL*, H. SUN, C. ZHOU, F. E. JENSEN;
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Abstract: Early-life seizures are highly prevalent, and can be associated with autistic-like behavior and spontaneous recurrent seizures in humans and rats. We previously showed a rapid, persistent increase in AMPAR function in CA1 hippocampal neurons after hypoxic seizures (HS) at postnatal day (P)10 in rats, and that this, like long-term development of spontaneous recurrent seizures and autistic-like behavior, could be prevented by acute, brief treatment with the AMPAR antagonist NBQX. Recently, we demonstrated that expression of synaptic GluA2 in CA1 decreases in rats 48hr post-HS, concurrent with functional changes that include LTD attenuation and increased Ca2+ responses. NBQX treatment prevented these functional changes, though the mechanism underlying the efficacy of NBQX on the rescue of these changes remained unclear. Given the critical role of GluA2-lacking AMPARs in seizure-induced changes in the developing hippocampus, we examined whether NBQX altered synaptic GluA2 levels 48hrs post-HS. Confocal immunohistochemistry showed that GluA2/synapsin colocalization was significantly higher in rats treated with NBQX administered immediately, 12, 24, and 36hrs post-HS than in vehicle-treated post-HS littermates (p=0.01, n=14 fields from 6 rats/group) and not significantly different from littermate controls (p=0.719, n=7 fields from 4 rats). NBQX treatment did not affect GluA2 in controls (p=0.719, n=7 fields from 4 rats). Demonstrating that this GluA2 rescue had a functional consequence, fura-2 imaging showed that percent change in peak response to KA before and after GluA2-lacking AMPAR blocker NASP incubation was significantly decreased in vehicle-treated animals (p<0.0001; n=6 slices from 4 rats; 10-26 cells/slice). Conversely, following *in vivo* NBQX treatment, NASP does not significantly alter the Ca2+ response (p=0.79; n=6 slices from 4 rats; 10-26 cells/slice). To confirm that *in vivo* NBQX treatment post-HS are functionally dampening CP-AMPARs, we used whole cell patch clamp to measure rectification ratios in adjacent slices. We observed restoration of rectification levels following NBQX treatment in slices taken from the same animals as the Ca2+ imaging (n=8 HS+Veh; n=7 HS+NBQX). Taken together, these results suggest that GluA2 expression is a modifiable factor that is critical to the effects of seizures on developing networks, and may provide a mechanism of action whereby NBQX rescues previously described seizure induced later life behavior deficits and epileptogenesis. Further evaluation of the subcellular mechanisms regulating GluA2 trafficking will be required to fully understand these effects.

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Poster

492. Epilepsy Network and Synaptic Mechanisms

Location: Hall A
The brainstem is an independent generator of febrile seizures

Authors: M. PUSKARJOV¹, A. POSPELOV¹, A. YUKIN¹, M. S. BLUMBERG², *S. BÄCK¹,
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Abstract: Febrile seizures (FS) are the most common type of convulsive events in children. The mechanisms underlying FS have been extensively studied in animal models. A widely held assumption is that FS are limbic in origin, and that the generalization of seizures, which is typical of FS, is caused by invasion of the brainstem by the initial limbic seizure activity. Based on a rat model of FS where 13-day-old rats are exposed to hyperthermia (Schuchmann et al., 2006 NatMed 12:817), we examined whether the brainstem by itself is able to generate tonic-clonic seizures. Rats with their forebrain surgically isolated from the brainstem (precollicular transection; Todd et al., 2010 BehavNeurosci 124:69) were exposed to hyperthermia. Sham-operated littermates were used as controls. Hyperthermia caused a similar increase in breath rate and blood pH between the two groups, which is important as we have previously shown that respiratory alkalosis is a major trigger of experimental FS (Schuchmann et al., 2006). Strikingly, we found that in transected animals tonic-clonic seizures occurred at a shorter latency (~7 min vs ~10 min) and with a lower temperature threshold (41.2 °C vs 43.4 °C) than in the sham-operated controls. We have shown before that FS are enhanced by low doses (150 µg/kg, i.p) of diazepam (DZP), whereas high doses (2.5 mg/kg, i.p) have a seizure-suppressing effect (Ruusuvuori et al., 2013 EMBOJ 32:2275). The latter is most likely caused by suppression of breathing by DZP and the consequent prevention of the FS-triggering respiratory alkalosis (Ruusuvuori et al., 2013). In line with this, the tonic-clonic seizures in the transected animals were not affected by 150 µg/kg DZP, while 2.5 mg/kg had a marked anticonvulsant effect. Systemic administration of kainate is known to produce limbic seizures which are thought to generalize after recruitment of brain stem networks. Notably, following kainate injection (3 mg/kg) in the transected rats, violent tonic-clonic seizures readily took place and, as was the case with the experimental FS, they had a shorter latency to onset than in the sham-operated controls. Our work suggests that the role of endogenous brainstem activity in generalized seizures should be re-evaluated in standard experimental models of limbic seizures. Moreover, the immobility that is commonly seen in rodents during cortical electrographic seizures may reflect a suppression of brainstem activity by hippocampal/cortical seizures (Ruusuvuori et al., 2013). This suggestion gains support from the
heightened proneness to tonic-clonic convulsions in the precollicularly transected animals in the two seizure models studied presently.

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Poster

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Title: Cardiac arrhythmogenic leaky ryanodine receptor 2 mutation increases cortical excitability and lowers threshold for spreading depolarization in mouse brain

Authors: *I. AIBA, J. L. NOEBELS;
Dept. of Neurol., Baylor Col. of Med., Houston, TX

Abstract: The ryanodine receptor (RYR) is an endoplasmic intracellular Ca2+ channel that, upon activation, releases Ca2+ into the cytoplasm. Mutations in the RYR2 subtype are found in congenital heart disease patients at risk of sudden cardiac death and victims of sudden unexpected death in epilepsy (SUDEP). Most pathogenic RYR2 mutations are gain-of-function, forming “leaky” channels that result in abnormal intracellular Ca2+ handling in cardiomyocytes and cardiac arrhythmias. Since RYR2 is also expressed in neurons, sudden death could also reflect CNS dysfunction. We recently showed that spreading depolarization (SD) is implicated in silencing brainstem cardiorespiratory activity in mouse SUDEP models. Since ryanodine receptor modulation of neuronal excitability has not been well studied, we investigated whether leaky RYR2 mutations might affect network excitability and SD threshold. We studied transgenic mice (4-8 weeks old) carrying the Ryr2 R176Q mutation, a dominant mutation identified in arrhythmogenic right atrial dysplasia patients. In chronic EEG/EKG recordings, heterozygous mice (R176Q+) showed periodic cardiac arrhythmias and cortical spikes. Rare seizures were observed. The cortical SD threshold was evaluated in vivo under urethane anesthesia by topical application of 1M KCl in a cranial window and detected in DC recordings with surface silver electrodes. The number of SD’s triggered in R176Q/+ mice was significantly
increased compared to WT control mice, suggesting a lowered SD threshold in the R176Q/+ mice. The SD phenotype of R176Q/+ mice was also characterized in acute cortical slices. Microinjection of 1M KCl triggered SD in both WT and RQ/+ slices. The SD propagation rate was significantly faster in slices from the heterozygous RQ mice. In other experiments, SD was generated by continuous exposure to modified artificial cerebrospinal fluid (ACSF) lacking Mg2+. Exposure to Mg2+ free solution resulted in spontaneous SD’s as well as seizure-like activity. The number of SDs was higher in slices prepared from mutant mice than WT. On the other hand, the incidence of seizures was similar. SD threshold was also tested in the oxygen glucose deprivation (OGD; 0% O2, 2 mM glucose) exposure model. Exposure to OGD solution reliably triggered SD, and the latency to SD onset was significantly faster in R176Q/+ slices. Together, these results consistently indicate that leaky RYR2 channels facilitate SD generation, and support the hypothesis that RYR2 mutations found in people at risk of sudden cardiac death may increase risk of neurogenic death by lowering the threshold for SD.

Disclosures: I. Aiba: None. J.L. Noebels: None.

Poster

492. Epilepsy Network and Synaptic Mechanisms

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Title: Epileptic encephalopathy mutations in KCNQ2 disrupt expression and function of KCNQ channels and affects hippocampal excitability

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Abstract: KCNQ channels are voltage-gated potassium channels composed of KCNQ2 and KCNQ3 subunits. Highly concentrated at the axonal surface where action potentials initiate and propagate, they potently inhibit repetitive and burst firing of action potentials, which is the hallmark for neuronal hyperexcitability leading to seizures. Indeed, KCNQ2 and KCNQ3 mutations cause benign familial neonatal convulsion and myokymia. We have shown that some
of these mutations impair axonal enrichment of KCNQ channels by disrupting KCNQ2 binding to calcium-binding protein calmodulin, and blocking their trafficking from the endoplasmic reticulum (ER) to the axonal surface (Chung et al, 2006; Cavarett et al., 2014). Recently, de novo mutations in KCNQ2 were discovered in patients with epileptic encephalopathy, severe symptomatic drug-resistant epilepsy with severe psychomotor retardation. Half of these mutations were found in the KCNQ2 C-terminal tail that interacts with calmodulin. Here, we show that epileptic encephalopathy mutations in KCNQ2 disrupt the axonal enrichment of KCNQ channels by reducing KCNQ2 binding to calmodulin and KCNQ3, leading to their ER retention. Importantly, expression of wild-type KCNQ2 but not epilepsy mutant KCNQ2 in hippocampal neurons suppresses their intrinsic excitability, suggesting that epilepsy mutant KCNQ2 is functionally defective. The ER resident chaperones mediate the retention of misfolded proteins in the ER and this process requires calcium in the ER lumen. Interestingly, depletion of calcium from the ER lumen could promotes the trafficking of wild-type and some epilepsy mutant KCNQ channels from the ER back to the axonal surface. These results provide an insight into the etiology of KCNQ2-associated epileptic encephalopathy, and may lay a firm foundation for targeting the ER chaperones as a possible therapy for epilepsy.


Poster

492. Epilepsy Network and Synaptic Mechanisms

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

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Topic: C.07. Epilepsy

Support: CRESim & Epi-CRESim Project

Title: Dynamic changes of depolarizing GABA in a computational model of epileptic brain: Insight for DRAVET syndrome

Authors: *P. BENQUET*¹, P. KURBATOVA², F. WENDLING¹, A. KAMINSKA³, C. CORNU⁴, G. PONS⁵, P. NONNY⁴, O. DULAC⁴, A. ROSATI⁵, R. GUERRINI⁶, R. NABBOUT⁶, C. CHIRON³;

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Florence, Firenze, Italy; UMR U1129, Inserm-University Paris Descartes-CEA, Paris, Paris, France

Abstract: Early infant onset epilepsies might be a consequence of abnormal reemergence of depolarizing GABAA current during postnatal maturation of the central nervous system. To study the impact of the contribution of depolarizing GABA in distinct pattern of EEG activity, we used a lumped-parameter approach lying at the level of cortical neuronal population used to represent the generation of spontaneous EEG activity. The model includes one sub-population of pyramidal cells and two sub-populations of interacting interneurons: perisomatic-projecting interneurons (basket-like) with fast synaptic kinetics GABAA (fast, I1) and dendritic-projecting interneurons with slow synaptic kinetics GABAA (slow). Basket-like cells were interconnected to produce mutual inhibition (I1→I1). This model (Molae-Ardekani et al., 2010) was modified to account for a number of neurobiological hypotheses. The firing rate of interneurons was adapted to mimic the genetic alteration of voltage gated sodium channels often found in Dravet syndrome, SCN1A−/−. We implemented the mechanism referred to as “dynamic depolarizing GABAA” mediated post-synaptic potential in the model, as a number of studies reported that the chloride reversal potential can switch from negative value to more positive value depending on interneuronal activity. The “shunting inhibition” promoted by GABAA receptor activation was also implemented. Finally, the dose dependent effect of benzodiazepine and Stiripentol on the generated field potential was considered in the model. We found that for unchanged parameters configurations of the computational model, the simple increase of the proportion of depolarizing GABAA mediated IPSP (I1→I1 and I1→P) is sufficient to switch the EEG activity from background to (1) interictal isolated polymorphic epileptic spikes, (2) to fast onset activity (chirp-like activity),(3) to seizure like activity and (4) seizure termination. Different morphologies of interictal spikes and patterns of seizures that we observed in several DS patients were reproduced in the model by tuning the amount of depolarizing GABAA postsynaptic potential. Finally, we implement the effect of two pharmacological product, benzodiazepine and stiripentol that were able to block dose-dependently this seizure-like activity, and found a synergic effect when these two drugs were combined.


Poster

493. Human Clinical Neurophysiology

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM
High frequency oscillations (HFO) are considered biomarkers of the seizure onset zone and its determination is crucial for the treatment of drug resistant epilepsy (DRE). It has been found that the most frequent DRE is the mesial temporal lobe epilepsy (MTLE) and its resistance has been proposed to be due to hippocampal sclerosis. Also, it has been found that there exist an increased ratio of fast ripples / ripples in patients with drug resistant MTLE and hippocampal sclerosis compared with those without the last one. Then in order to address this, we analyzed interictal electroencephalograms recorded with deep brain electrodes from patients with DRMTLE included in a protocol for deep brain stimulation. Preliminary results confirm that the ratio of fast ripples / ripples is increased in patients with hippocampal sclerosis, and they correlate with the side of the hippocampal sclerosis as reported from magnetic resonance image and spectroscopy. One case of a patient with bilateral onset zone and unilateral sclerosis is also discussed.
**Authors:** *S. KOCHEN*¹, B. GORI², M. GRANADO³, A. BLENKMANN²;  
¹Epilepsy Ctr., ¹Conicet, Capital Federal, Argentina; ³Univ. of Buenos Aires, capital federal, Argentina

**Abstract:** We studied the spatiotemporal scale of focal epilepsy, the dynamics of its interaction, and the spread of epileptogenesis. We used wide-bandwidth electrophysiological intracranial recordings using clinical macro- and research microelectrodes in patients with epilepsy. All seizures were identified by an epileptologist from the macroelectrode recordings. We analyzed 23 spontaneous seizures of two patients with insular epilepsy (IE) and frontal epilepsy (FE). For further analysis, we only included seizures (n=11) that presented a local field potential (LFP) with single unit activity during ictal period and at least within 15 minutes before seizure onset. LFP and single units were recorded across multiple days using Ad Tech (40 um width) microwires. Single units were classified by an automated cluster identification program (wave_clus, MATLAB). When a given unit was identified as a single cell across multiple seizures and multiple days, results were manually grouped based on similarities in waveform, spike widths, spike density, and interspike interval histogram. The firing rate (FR) during basal, preictal, ictal, and postictal periods was calculated as the total number of action potentials per unit of time. In the IE case, microelectrodes were localized within the epileptogenic zone (EZ) in the posterior insula. However, seizures recorded with macroelectrodes were not simultaneously observed with adjacent microelectrodes in all cases. LFP with single unit recording during seizures showed unstructured activity different to basal period, but did not present epileptiform discharges. The FR either remained constant or decreased. In the FE case, microwires were localized in the hippocampus, outside the EZ. When seizures spread to the hippocampal area, the macroelectrode recorded epileptiform discharges that were simultaneously observed on LFP, showing sharp waves higher than 2 Hz. In this case, a marked increase in FR was observed in all seizures. The areas involves directly in the seizure or its propagation that showed hypersynchronous discharges on LFP, presented an increased in FR. While the LFP did not show epileptiform discharges in spite of being part of the EZ, the FR only show low-level, and unstructured activity. These findings could have important implications for how we localize seizure activity and how we map its propagation.

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**Poster**

**493. Human Clinical Neurophysiology**

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**Topic:** C.07. Epilepsy

**Title:** Evidence for the implant effect in chronic ambulatory human ECoGs

**Authors:** *S. ARCOT DESAI, F. T. SUN, T. K. TCHENG, M. J. MORRELL;* NeuroPace, Mountain View, CA

**Abstract:** Electrocorticographic records (ECoGs) collected from chronically implanted NeuroPace depth and/or cortical strip leads in 126 subjects with epilepsy were analyzed. All subjects were participating in a double-blind, randomized, sham-stimulation controlled trial of a responsive neurostimulator (RNS® System, NeuroPace, Inc.) as an adjunctive treatment for medically intractable partial onset seizures. To be included in this analysis, subjects must have had at least 100 interictal ECoGs stored over at least one year since implantation of the RNS® Neurostimulator and NeuroPace leads. The analysis included an average of 763 scheduled ECoGs per subject, for a total of 96,162 ECoGs. Overall power and normalized power within delta, theta, alpha, beta, low gamma, and high gamma frequency bands, and spike rate were measured on each channel of the scheduled ECoGs. Month-to-month differences were assessed for each patient ECoG channel, and group statistics were calculated by averaging the within-channel results. There were significant month-to-month changes in overall power, normalized power within frequency bands, and spike rate that were most pronounced in the first 3 to 5 months after implant. There was a significant change in over half (55%) of all the ECoG channels from the first to the second month (p < 0.05, two-sample t-test), including 68% of the channels recorded from strip lead electrodes and 47% of the channels recorded from depth lead electrodes. After 5 months, the overall power became more stable, with significant month-to-month changes seen on average in less than 20% of the channels recorded from depth lead electrodes and less than 25% from strip lead electrodes. Similar patterns of changes were observed with normalized power within frequency bands as well as for spike rate. While power-related changes may be predominately related to changes at the electrode-tissue interface that impact impedance, spike rate changes suggest that there may be additional neurophysiological changes in the first few months following implantation - “the implant effect”. Hence the ECoG data collected in the 3 to 5 months after implantation of depth or subdural electrodes are not stable and may not be representative of the chronic state.

**Disclosures:** S. Arcot Desai: A. Employment/Salary (full or part-time); NeuroPace, Inc. F.T. Sun: A. Employment/Salary (full or part-time); NeuroPace, Inc. T.K. Tcheng: A. Employment/Salary (full or part-time); NeuroPace, Inc. M.J. Morrell: A. Employment/Salary (full or part-time); NeuroPace, Inc.

**Poster**

493. Human Clinical Neurophysiology
Title: Delay differential analysis: a framework for the analysis of large-scale epileptic electrocorticography recordings


Abstract: High density electrocorticogram (ECoG) electrodes are capable of recording neurophysiological data with high temporal and reasonable spatial resolution. Such recordings are a window to understanding how the human brain processes information and subsequently behaves in healthy and pathologic states. At present, many of the computational methods utilized in the analysis of ECoG recordings are strictly linear, require significant pre-processing, and fail to provide high-level information with respect to the state of the neurological system. In the following study, we describe and implement delay differential analysis (DDA) for the characterization of ECoG data obtained from human patients with intractable epilepsy. DDA is a time domain analysis framework based on embedding theory in nonlinear dynamics. An embedding reveals the nonlinear invariant properties of an unknown dynamical system (here the brain) from a single time series (ECoG signals). The DDA embedding serves as a low-dimensional nonlinear functional basis onto which the data are mapped. Since the basis is built on the dynamical structure of the data, preprocessing of the data, e.g. filtering, is not necessary. DDA yields a low number of features (four or less), far fewer than traditional spectral techniques. This greatly reduces the risk of overfitting and improves the method's ability to fit classes of data. One single three term DDA is shown to qualitatively discriminate between different neurologic states and epileptic events for a set of 13 patients from the raw ECoG data. Singular value computation across the feature space is shown to delineate global and local dynamics. The global and local dynamics differentiate electrographic and electroclinical seizures while also providing insight into a highly localized seizure onset and diffuse seizure termination. Thus, DDA is shown as a new form of computational analysis for ECoG data obtained from the epileptic patient.
Disclosures: J. Weyhenmeyer: None. C. Lainscek: None. S.S. Cash: None. T.J. Sejnowski: None.

Poster

493. Human Clinical Neurophysiology

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

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Support: Academy of Finland 253130

Academy of Finland 256472

Academy of Finland 1266745

Academy of Finland 266402

European Union Seventh Framework Programme (FP7/2007-)

Title: Robust long-range high-gamma phase synchronization in human cortex

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Abstract: Mammalian cortical activity is thought to exhibit long-range synchronization only in frequency bands up to ~100 Hz. While high-gamma-frequency band (100-250 Hz) activities have been observed and found to be associated with behaviourally relevant neuronal processing and epileptiform activity, they have been thought to be strictly local. We report here that long-range synchronization in the high-gamma band is, in fact, a robust and widespread physiological phenomenon in the human brain. Stereo-electroencephalography (SEEG) is a routine clinical method used in presurgical identification of epileptic foci by intra-cerebral recording of local field potentials in subjects suffering from refractory epilepsy. SEEG is emerging as an increasingly used tool in neuroscience research because it yields spatially and temporally accurate, gold-standard-quality like recordings of local field potentials (LFPs) in awake humans. We used a cohort of 22 subjects for which the SEEG electrode contacts were automatically
localized with sub-millimetre accuracy in the individual cerebral volume. We quantified phase and amplitude correlations between electrode contacts located in cortical or subcortical grey matter. These LFP recordings were locally re-referenced to closest contacts in the underlying white matter. We found that, as expected, the phase and amplitude correlations decayed as a function of frequency and distance up to approx. 100 Hz. This decay, however, was reversed after ~100 Hz and exhibited a peak in the high-gamma frequency range at around 200 Hz. To the best of our knowledge, this is the first observation of long-range (3-10 cm) phase synchrony in the high-gamma frequency band. We show that these interactions were not attributable to volume conduction, specific referencing schemes, technical or epileptic spike artefacts, harmonics of line noise, or volume-conducted muscular activity signals. We found also that the interactions between the deep and superficial cortical layers exhibited distinct spectral profiles and while this long-range synchronization was salient between subcortical and cortical structures, it was also significant among cortical regions. Importantly, high-frequency gamma synchrony was observed not limited to cortical regions in or connected with the epileptic zone but rather was equally characteristic to putatively healthy parts of the neocortex. These data suggest that long-range phase correlations among high-gamma-band oscillations are a genuine physiological phenomenon and are not attributable to artefactual sources or limited to the network connected to the epileptic zone.


Poster

493. Human Clinical Neurophysiology

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Topic: C.07. Epilepsy

Support: NINDS RO1-NS062092
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ECOR-MGH Research Scholars Award
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Title: Characteristics of seizure termination in primary and secondarily generalized seizures
Authors: *M. BORZELLO\textsuperscript{1}, A. MAHESHWARI\textsuperscript{2}, C. CHU\textsuperscript{1}, M. KRAMER\textsuperscript{3}, B. M. WESTOVER\textsuperscript{1}, S. S. CASH\textsuperscript{1};
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Abstract: The vast majority of seizures end spontaneously. Understanding the mechanisms underlying this phenomenon would be useful not only in understanding what happens when a seizure becomes prolonged, evolving into status epilepticus, but also in designing effective clinical interventions. The purpose of this study is to compare the termination patterns of two canonically distinct categories- seizures with electrographically focal onset that become secondarily generalized (sGTC) and seizures appearing to have electrographically generalized onset as seen in idiopathic generalized epilepsy (IGE) or childhood absence epilepsy (CAE). We examined the termination pattern of a total of 167 seizures in 57 patients- 71 with focal onset that secondarily generalized (scalp EEG: 45, intracranial EEG: 26) and 96 seizures with generalized onset (IGE:10, CAE: 86). Our results expose characteristics that occur at the end of each type of epileptic episode suggesting a common final mechanism. We found that while the two classes of seizure onset under investigation - focal and generalized - seem to terminate in their own unique way, there are common characteristics between the two types. Analyzing secondarily generalized seizures from scalp and intracranial recordings, we identified a conspicuous electrographic motif- a “burst suppression pattern” similar to those in anesthesia recordings. With scalp data, we observed robust synchrony amongst channels and a simultaneous end in which seizure activity terminated in all leads at the same time. Activity from scalp electrodes was generally more homogeneous than the intracranial electrodes. The length of suppressions significantly increased toward seizure end while the length of bursts showed no significant change. Furthermore, we analyzed generalized seizures from scalp data and found a similar burst suppression pattern, though not identical to that of the focal-onset seizures. In contrast to both IGE and sGTC, seizures from patients with CAE showed a general trend of slowing and decreased amplitude at seizure end but no clear burst-suppression pattern. These characteristic ending patterns can be used to constrain mechanistic models of spontaneous seizure cessation and inform ideas about new therapies designed to curtail seizure intensity or end them altogether.

Disclosures: M. Borzello: None. A. Maheshwari: None. C. Chu: None. M. Kramer: None. B.M. Westover: None. S.S. Cash: None.

Poster

493. Human Clinical Neurophysiology

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM
Title: Reactivation of neuronal ensemble spiking patterns during human focal seizures

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Abstract: The collective dynamics of neuronal ensembles during focal seizures is a fundamental
problem in neuroscience and in the development of new therapies for pharmacologically
intractable epilepsy. It remains unknown whether neuronal subsets are randomly recruited to
engage with different patterns of activity in every seizure, or whether reoccurrence of seizures
leads to reactivations of similar patterns in the same neuronal ensemble. By contrast to a random
recruitment hypothesis, here we characterize patterns of neuronal reactivations that are
stereotypical and highly reproducible across propagated seizures within the same patient.
Ensembles of single neurons were simultaneously recorded via 96-microelectroded arrays
implanted in the neocortex of patients undergoing neuromonitoring prior to resective surgery. In
a few patients, we were able to record the same neuronal ensemble across different seizures. Two
complementary statistical modeling approaches were applied to characterize the collective
dynamics in recorded multivariate neuronal point processes and capture the recurring network
patterns. One approach is based on a low-dimensional hidden linear dynamical system that drives
the firing rates of individual single-neurons in the ensemble. The other approach couples
neurons' firing rates to the ensemble activity through a dense network of effective connections
and a newly introduced mean-field coupling. We find that both models could predict single-
neuron firing equally well: Cross-validated prediction scores based on the area under ROC
curves ranged from 0.7 to 0.9 for two types of seizures with qualitatively different dynamics.
Furthermore, predictions of both models were partially correlated, indicating that models
captured similar dynamical features of the spiking patterns. We hypothesize that a combination
of both models could further improve prediction. Both models provide a generative mechanism
for the underlying seizure dynamics that could not otherwise be derived from simpler,
Our findings indicate that the reactivation of the stereotypical patterns of neuronal activity during seizures can be captured by the evolution of collective dynamics in the recorded ensemble. The reoccurrence of activity patterns in neuronal ensembles points to the existence of temporally stable, effective microcircuits that are reactivated during seizures. Furthermore, compact statistical descriptions of high-dimensional neural recordings such as those presented here are a major step towards better algorithms to detect and control seizures in people with pharmacologically resistant seizures.

**Disclosures:** F. Gerhard: None. S.S. Cash: None. W. Truccolo: None.

**Poster**

**493. Human Clinical Neurophysiology**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 493.08/F25

**Topic:** C.07. Epilepsy

**Support:** NSF 1406556

**Title:** Identification of epileptogenic network using intrinsic evoked potentials

**Authors:** *S. KARUNAKARAN*¹, C. M. KADIPASAOGLU², G. P. KALAMANGALAM¹, B. AAZHANG³, N. TANDON³;

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**Abstract:** It is becoming increasingly accepted that seizures are a manifestation of aberrant network function involving disseminated cortical and subcortical brain regions rather than a single epileptogenic focus. Identification of patient-specific epileptogenic networks is a crucial first step in modulating these networks to decrease seizure probability. Characterizing the seizure network involves identifying anatomical regions (i.e. nodes) of increased excitability, and estimating the intensity and direction of information flow between these nodes. Intracranial recordings in patients undergoing pre-surgical evaluation for medically refractive epilepsy provide a unique opportunity to evaluate and isolate seizure networks. Traditionally, cerebral connectivity is estimated using cortico-cortical evoked potentials (CCEPs) in response to external electrical stimulation. CCEPs have also been used to delineate ictal-onset regions and regions of early and late seizure propagation. Similar to these CCEPs, responses to endogenous inter-ictal spikes provide an indirect measure of functional connectivity. We propose to use evoked potentials from endogenous inter-ictal signals, particularly inter-ictal spikes to identify
and visualize functional connectivity among brain regions. We hypothesize that inter-ictal spikes will evoke strong responses from sub-regions of the networks involved in seizure propagation. Three hundred spontaneously occurring epileptiform spike discharges were used to align recordings from other cortical regions in two patients. We found that the majority of electrodes having large average responses to inter-ictal spikes overlay regions exhibiting rhythmic discharges during seizures. In addition to nearby regions that are expected to have large responses, regions distant from the reference region also showed large responses suggestive of aberrant connectivity that likely represents the epileptic network. In comparison to the stimulation paradigm for obtaining CCEPs, which is time-consuming and determined by clinical needs, intrinsic evoked potentials (IEPs) from endogenous spikes utilize the routinely collected inter-ictal data. The precise patient-specific information obtained using these methods can then be used to target the most susceptible nodes by ablative or neuromodulation strategies.


Poster

493. Human Clinical Neurophysiology

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 493.09/F26

Topic: C.07. Epilepsy

Support: KTIA_NAP_13-1-2013-0001

Title: Evaluation of the components of the cortico-cortical evoked potentials with single and paired pulse subdural electrical stimulation in epilepsy patients

Authors: B. HAJNAL1,2, *L. ENTZ1,2, E. TOTH3, I. ULBERT4, D. FABO1, L. EROSS1;
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Abstract: Cortical electrical, and transcranial magnetic stimulation (CES and TMS) can be reliably used in the investigation of cortical excitability, localization of the seizure focus and mapping of the cortico-cortical networks. Moreover these modalities can be effective in the therapy of drug-resistant epilepsy. Paired-pulse TMS study delivering preconditioning and test stimulus with various ISIs showed short interval inhibition (1-6ms), a short interval excitation (8-30ms) and a long interval inhibition (50-200 ms). Recently our group found that CES applied on
subdural electrodes resulted in a single wave of slow oscillation (SO) regardless of the vigilance state of the patient. Physiological mechanisms underlying electrophysiological changes observed during cortical electrical stimulation is barely known in humans, although there are presumptions regarding to the excitatory and inhibitory neuronal mechanisms during paroxysmal discharges. We studied single (n=10) and paired-pulse (n=4) electrical cortical stimulation on drug-resistant epilepsy patients implanted with subdural grid and strip electrodes, and laminar multielectrodes (24 contact, 200\mu m). We applied brief single (10mA, 0.2ms, 0.5Hz) and paired (ISI: 6.6, 10, 20, 30, 40, 50, 100, 200, 500, 1000ms, on the best single response electrode-pair) current pulses on adjacent contacts of the grid and strip electrodes. Various phases of cortico-cortical evoked potentials (CCEP) were analyzed using custom scripts in Matlab. We found P1, N1, P2, N2 components with average latencies of 12, 30, 60, 160 ms respectively. The larger was the distance between stimulating and recording electrodes on the same gyrus, the longer latency components were evoked only. The laminar profile showed surface current sink and layer IV source for N1, surface source and layer IV sink for P2, and a wide source for N2 in the middle layers. Amplitude difference of P1-N1, N1-P2, P2-N2 was correlated with ISI. Values measured at 2000ms ISI was used as baseline. We found similar N1-P2 curve characteristics in 3 out of the 4 patients evaluated with an excitation at ISI 7 and 10ms, inhibition at ISI 20-50ms and a long interval excitation at ISI 200 and 500ms. We were able to describe the laminar profile of different phases of the CCEPs, and succeeded to identify excitatory (P1, N1) and inhibitory (P2, N2) components. Previously reported evoked SO was identical to N2 in the present abstract. In our paired pulse stimulation setting we demonstrated these inhibitory and excitatory effects on the second CCEP response.

**Disclosures:** B. Hajnal: None. L. Entz: None. E. Toth: None. I. Ulbert: None. D. Fabo: None. L. Eross: None.

**Poster**

493. Human Clinical Neurophysiology

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**Topic:** C.07. Epilepsy

**Support:** NINDS Grant R01NS079533  
DVA Grant RX000668-01A2

**Title:** Robust threshold estimation for detection of discrete neural events in long-term human recordings
Abstract: The detection of neural events over long time periods introduces new challenges to an already complicated signal detection problem, e.g. detecting spike-wave discharges from local field potentials (LFPs) or action potentials from broadband recordings in human neocortex. In relatively artifact-free data, the background noise tends to be stable over hours/days due to the stability of resistance/capacitance features of electrodes in these time scales. However, current state-of-the-art methods for estimating detection thresholds (e.g., methods based on standard deviation (SD) of the noise) are not robust to fluctuations in ongoing neural activity and thus cannot reliably detect discrete neural events. Here, we examine an approach for estimating the detection threshold for individual electrodes based on maximizing the log-likelihood of a probabilistic mixture model, in which a separate model is assigned for the background noise and neural signal. In particular, we consider the case of a mixture of an Exponential-Gaussian density function to fit the highly skewed noise distribution, and a Gaussian density function to fit the target neural signal distribution. Under significant fluctuations in the level of neural activity in our datasets, the parameters of the Gaussian density function (mean and variance) and the mixture weight varied substantially over time, while the parameters of the Exponential-Gaussian density function, which identifies the distribution of the background noise, tended to remain almost constant. A simpler two-class Gaussian mixture model (GMM) failed to provide reasonable estimates of the detection threshold due to the skewness of the noise distribution. In comparison with the two-class GMM, the proposed mixture model significantly reduced the Kullback-Leibler divergence between the empirical and estimated signal distribution. Visual inspection of the data indicated that the new proposed approach led to more stable estimation of the detection threshold and fewer false positives in comparison to previous SD and two-class GMM methods.

Disclosures: M. Aghagolzadeh: A. Employment/Salary (full or part-time); Brown University. F. Gerhard: A. Employment/Salary (full or part-time); Brown University. W. Truccolo: A. Employment/Salary (full or part-time); Brown University.
Title: Spectrum of seizures in patients with acute encephalopathy, biphasic seizures, and late reduced diffusion

Authors: Y. MURATA1, *K. MURAMOTO3, F. OKUTANI2, N. HAMADA1, Y. HATA4, T. YAMAGAMI1;
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Abstract: Seizures in patients with acute encephalopathy, biphasic seizures, and late reduced diffusion (AESD) are varied. The aim of the current study was to evaluate how the duration of initial and/or subsequent seizures interacts with the occurrence of AESD and prognosis of pediatric emergency patients with their first febrile seizures. Seventy-three patients (M:F [39:34]; age range, 0-14 years; mean years, 2.8 years), who were evaluated in our pediatric emergency department with their first febrile seizure, were retrospectively reviewed. Prolonged seizures > 30 min in length (status epilepticus), clusters of complex partial seizures < 30 min in length, and brief seizures occurred in 33, 5, and 35 patients, respectively. All 73 patients had brain MRI including diffusion weighted image (DWI) within 24 h of arrival at our hospital. Based on clinical and electroencephalogram (EEG) findings, 10 (M:F [5:5]; age range, 0-6 years; mean age, 2.5 years) of 33 patients (M:F [18:15]; age range, 0-10 years; mean age, 2.3 years) with prolonged seizures resulted in AESD; none of the remaining 40 patients with initial seizures < 30 min developed AESD. Six of 10 patients had a subsequent seizure of shorter duration than the initial seizure, 2 patients had clusters of complex partial seizures for several days, and 2 patients did not have a secondary seizure. One of the 2 patients without a secondary seizure had subcortical diffuse abnormalities in the initial MRI, a high fever lasting several days with delirium, and had the worst prognosis of the 10 patients. Nine of 10 patients had no abnormalities on the initial MRI, and all 9 patients had MRI abnormalities between 2 and 5 days after initial MRI. Subsequent seizures were not related to the prognosis in the 9 patients. Appropriate medical care for the first onset febrile seizure < 30 min could prevent subsequent seizures and the occurrence of AESD, although prolonged seizures could not be avoided.


Poster

493. Human Clinical Neurophysiology

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 493.12/F29
Topic: C.07. Epilepsy

Title: Automated ictal and postictal behavioral testing of epilepsy patients

Authors: G. Touloumes1, W. Chen1, A. Sivaraju1, R. Khozein1, E. Morse1, C. Cunningham1, L. J. Hirsch1, H. Blumenfeld2; 1Neurol., 2Neurology, Neurobiology, & Neurosurg., Yale Univ. Sch. of Med., New Haven, CT

Abstract: Impaired consciousness in epilepsy is highly detrimental to patients’ quality of life, but is difficult to quantitatively characterize in a reliable manner. Information about impaired consciousness or responsiveness during seizures guides decisions about driving safety and helps determine the severity of seizures for surgical decision making. In previous studies, we developed and validated a prospective responsiveness in epilepsy scale (RES) which yielded standardized classifiers of patient cognition levels during seizures. Up to now, we relied on trained hospital personnel to sit at the bedside continuously in order to administer the RES to patients during seizures. Since this process was both error-prone and prone to missing seizures entirely, our aim was to fully automate the RES using a combination of seizure detection and structured playback of RES video clips. The computerized version of the RES (“Robo-RES”) was created to eliminate errors made during testing, to capture more seizures, and to reduce the latency between seizure onset and onset of the RES questioning without sacrificing the comprehensive set of sensorimotor and cognitive functions of interest. To build the Robo-RES, we recorded videos of the RES questions and dynamically combined them in a PsychoPy experiment task to be viewed by patients immediately after seizure onset on an all-in-one PC mounted in the patient’s room. The Robo-RES testing is triggered either by a patient pushbutton event or by Persyst 12 automatic seizure detection from the clinical video/EEG monitoring computer. To test functionality of the system we simulated seizure events using pre-recorded video/EEG data from 14 seizures in 4 patients. The Robo-RES was able to correctly initiate automatic behavioral testing for 100% (14/14) of seizures with a mean time lag of 2.1 s and no false-positive test initiations. The user interface was fully integrated with and did not impede the clinical recording system. Further testing in the Yale Comprehensive Epilepsy Center Video/EEG monitoring unit will enable full implementation of the Robo-RES for patients as part of their clinical care. Automatic ictal and postictal behavioral testing of epilepsy patients has the potential to greatly enhance information obtained during video/EEG monitoring that will help guide decisions about seizure severity and therapeutic interventions for people with epilepsy.


Poster

493. Human Clinical Neurophysiology
**Title:** Mechanisms of seizure identified from causal connectivity inferred using directed information

**Authors:** *R. MALLADI*, G. KALAMANGALAM, N. TANDON, B. AAZHANG; ¹Dept. of Electrical and Computer Engin., Rice Univ., Houston, TX; ²Dept. of Neurol., ³Dept. of Neurosurg., Univ. of Texas Hlth. Sci. Ctr., Houston, TX

**Abstract:** Epilepsy is a common neurological disorder affecting nearly 1% of the world's population. The current treatments for epilepsy based on medication and surgical resection are not effective. Learning how seizures originate is crucial to develop next generation treatments for epilepsy. We analyzed the changes in causal connectivity over time in five epileptic patients to improve our understanding of seizures. The causal connectivity between electrodes implanted in an epileptic patient is estimated from electrocorticographic (ECoG) recordings using directed information (DI) from multiple shifted time-windows. Figure 1 plots the results of our analysis from a seizure of patient P1. The solid vertical black lines in Figure 1 represent the seizure start and end times as determined by neurologist. In addition, there is no significant seizure activity between 250s and 350s in this seizure. The mean and standard deviation of the average strength of the outgoing connections from all channels outside seizure onset zone (SOZ) and all channels in SOZ to the channels outside SOZ is plotted in Figure 1. The red and blue curves correspond to the connections from electrodes outside SOZ and within SOZ, respectively. It is clear from this figure that the electrodes outside SOZ become more synchronous during seizures implying that seizures occur when the regions outside SOZ become sufficiently hyper synchronous. We also observed a 'trigger' pulse (two small spikes in the blue curve) from the electrodes in SOZ to those outside at the beginning of the seizure activity. These trends are broadly observed in the remaining patients as well. In addition, we present the connections between the inferences made from our dynamic connectivity analysis in human patients and the seizure generation mechanisms observed in animal models of epilepsy. This could be the first step towards development of novel treatments for epilepsy. Figure 1: Dynamic causal connectivity of patient 1 inferred using directed information
Disclosures: R. Malladi: None. G. Kalamangalam: None. N. Tandon: None. B. Aazhang: None.

Poster
493. Human Clinical Neurophysiology

Location: Hall A
Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM
Program#/Poster#: 493.14/F31
Topic: F.01. Human Cognition and Behavior

Title: Hippocampal theta connectivity networks in normal and epileptic subjects demonstrated by magnetoencephalography

Authors: *A. Alhourani\(^1\), M. J. Randazzo\(^1\), T. A. Wozny\(^1\), E. D. Kondylis\(^1\), M. J. Ward\(^1\), A. Niranjan\(^1\), A. Bagic\(^2\), A. S. Ghuman\(^1\), R. Richardson\(^1\);
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Abstract: Understanding human memory relies, in part, on the study of the hippocampus. The subcortical location of the hippocampus, however, limits the neurophysiological sampling of hippocampal activity to electrophysiological recordings obtained from invasive depth electrodes or, indirectly, to metabolic changes observed using fMRI. Fortunately, recent developments in magnetoencephalography (MEG) analytic techniques are refining our ability to reliably record neural activity from subcortical structures. The objective of this study was to demonstrate the baseline hippocampal functional connectivity network using neural signals recorded with MEG. Eleven subjects (9 healthy controls and 2 subjects with unilateral mesial temporal lobe epilepsy (MTLE)) underwent 5 minutes of non-task-related MEG recordings with their eyes open. The signals were preprocessed and projected to source space onto a volumetric grid for the
hippocampal sources using Brainstorm MATLAB suite. Since multiple studies have linked the theta rhythm to hippocampal memory function and to information transfer to cortical areas, the weighted phase lag index (WPLI) for the theta frequency (4-8Hz) was averaged over 30 randomly selected, artifact-free 4 second epochs and averaged per subject. The same procedure was done on the empty room recording. Functionally connected regions were defined by selecting the regions showing WPLI values greater than 2.5 standard deviations from the mean (calculated using the Rayleigh statistic after subtracting the WPLI values for the empty room from the subject’s WPLI values to account for the imperfect inverse solution). The non-task, hippocampal theta network comprised parts of the limbic system and both the dorsal and ventral attention networks. In normal subjects, significant WPLI values were found between the anterior hippocampus and the ipsilateral anterior cingulate, orbitofrontal, anterior prefrontal, frontal eye field, premotor, superior parietal, fusiform, entorhinal and parahippocampal cortical areas. WPLI values indicated bilateral connectivity to temporopolar, occipital and middle temporal cortical areas. Patients with MTLE showed a similar pattern, however the epileptic hippocampus showed significantly reduced connectivity (p <0.01) compared to the non-involved side in both patients. Here we provide evidence that MEG can detect hippocampal-based networks using resting state data and that MTLE reduces hippocampal-cortical functional connectivity in the theta frequency band


**Poster**

494. Epilepsy Mechanisms

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 494.01/F32

**Topic:** C.07. Epilepsy

**Support:** NINDS P01NS045260-01

**Title:** Differential activation of calpain-1 and calpain-2 following kainate-induced seizure activity in rats and mice

**Authors:** *J. SEINFELD* 1, N. BAUDRY 1, X. XU 2, X. BI 1, M. BAUDRY 1;

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Abstract: Systemic injection of kainate produces repetitive seizure activity in both rats and mice. It also results in acute synaptic modifications as well as delayed neurodegeneration. The signaling cascades involved in both acute and delayed responses are not clearly defined. The calcium-dependent protease calpain is activated in various brain structures following kainate injection, although the precise involvement of the two major calpain isoforms, calpain-1 and calpain-2, remains to be defined. Calpain-2 selectively truncates the phosphatase PTEN, and calpain-1 and calpain-2 play opposite roles in NMDA receptor-mediated neuroprotection or neurodegeneration. In the present study, we determined KA-induced activation of calpain-1 and calpain-2 in hippocampus by analyzing changes in different calpain substrates, including spectrin, PTEN and drebrin in both rats and mice, both wild-type and calpain-1 knock-out (ko) mice. As PTEN is selectively cleaved by calpain-2 but not by calpain-1, decreases in PTEN levels detected by western blots or immunohistochemistry reflect selective calpain-2 activation. Similarly, changes in calpain substrates detected in calpain-1 ko mice are the results of calpain-2 activation. The results indicate that while calpain-2 is rapidly activated (less than 1 h) in pyramidal cells throughout CA1 and CA3, early calpain-1 activation (1-4 h) is restricted to selective population of parvalbumin-expressing interneurons in CA1 and CA3. As previously reported, neither calpain-1 or calpain-2 is activated in the dentate gyrus. In addition, calpain-1 knock-out mice exhibit increased long-term neurodegeneration in CA1 and CA3. These results suggest that calpain-1 and calpain-2 are differentially activated by seizure activity in different cellular populations in hippocampus. They strengthen the notion that calpain-1 activation is neuroprotective, while calpain-2 activation is neurodegenerative. Thus, a selective calpain-2 inhibitor could be beneficial for limiting the debilitative consequences of seizure activity.

Disclosures: J. Seinfeld: None. N. Baudry: None. X. Xu: None. X. Bi: None. M. Baudry: None.
Abstract: Epilepsy is one of the most common neurological disorders in humans, and the role of cerebellum in its physiopathology remains subject of study. Bergmann glia (BG) in the cerebellar cortex regulates the homeostasis of Purkinje cells (PC), whose axons targets the dentate and interpositus nuclei, which form the main cerebellar output to other structures in the central nervous system involved in Epilepsy. Sox-1 is a transcription factor expressed in BG and its binding to β-Catenin (βC) further inhibits the canonical Wnt pathway. It has been reported βC signaling is increased as the hippocampus receives repeated electrical stimuli and this is related with apoptosis of neurons. In the cerebellum, the recurrence of seizures results in PC death, although the mechanisms remain unclear. Here we present the expression of βC and the type of PC death in cerebellum of rats with seizures induced by amygdala kindling. We used Wistar rats separated into groups that received 3, 15, 45 stimuli and our controls. Once animals sacrificed, the cerebellum was processed for immunohistochemistry assay for βC, TUNEL and caspase-3. We found increased immunopositivity for βC expression in the nucleus and citosol of neurons in the 45-stimuli group compared to the controls and the rest of groups. TUNEL assay in the PC showed apoptosis in the 15-stimuli group and necrosis in the 45-stimuli group. Caspase-3 assay shows statically significant increased immunopositivity (p ≤ 0.05) in the 45-stimuli group compared to the others groups. We concluded that there are higher activity of βC associated with increased number of stimuli may be related with the presence of apoptosis and necrosis in the cerebellum treated with amygdala kindling. In this way we suggest this mechanism as a one possible explanation of PC death in epilepsy.


Poster

494. Epilepsy Mechanisms
**Location:** Hall A  
**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM  
**Program#/Poster#: 494.03/F34  
**Topic:** C.07. Epilepsy  

**Support:** This project was funded by the Mexican Health Research Fund, No. FIS / IMSS / PROT / G12 / 1124  

**Title:** The effect of honokiol and magnolol on the inflammatory response mediated by il1-β and cox-2 in a recurrent convulsive seizures model during the neonatal period  

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**Abstract:** Seizures generate an inflammatory response through the activation of microglia facilitating the epileptogenesis. The aim of this study was to test the Honokiol and Magnolol components of Magnolia officinalis (MG) on the inflammatory response in a epileptogenesis model induced by recurrent crises with AK. Method: Sham, kainic acid (AK), Celebrex (CLBx), Magnolia officinalis (MG): Male Sprague Dawley 10 PN, n = 48 rats were used and divided into four groups. The AK was administered at doses of 1.5 mg / kg ip from 10pn-14PN and treated with MG extract (300mg / kg) via esophageal from the 15th PN day and celecoxib (20mg / kg po) was tested as a control drug. Spontaneous seizure activity was evaluated at the 30th and 60th PN days through the evolution of motor behavior and the pro inflammatory COX2 proteins, IL1-β in temporal cortex, amygdala and hippocampus were quantified by Western Blot. In the Western Blot analysis, a significant reduction is observed P <0.05 over the expression of COX2, IL1β in amygdala in comparison with the hippocampus and temporal cortex at the 30th and 60th PN. This indicates that the MG treatment had anti-inflammatory effects and reduced the spontaneous crises over the SNC, indicating the role that inflammation has on the development of epilepsy. This project was funded by the Mexican Health Research Fund, No. FIS / IMSS / PROT / G12 / 1124  

**Disclosures:** A. Vega garcia: None. S. Orozoco suarez: None. A. Morales otal: None. L. Rocha arrieta: None. F. Dominguez aviles: None.
Title: A novel therapeutic strategy to reduce brain inflammation and injury after status epilepticus

Authors: *J. JIANG*¹, R. DINGLEDINE²;
¹James L. Winkle Col. of Pharm., Univ. of Cincinnati, Cincinnati, OH; ²Pharmacol., Emory Univ. Sch. of Med., Atlanta, GA

Abstract: Status epilepticus (SE) in humans causes high mortality and extensive morbidity in survivors. The only effective treatment currently is to stop the seizures quickly enough to prevent brain damage. However, reliance on acute therapies alone would be imprudent due to the required short response time, and more than one third of SE patients do not respond to current antiepileptic drugs. Thus, follow-on therapies that can be delivered well after seizure onset are needed. SE triggers a sequential of molecular, cellular and systemic alterations in the brain that often culminate in the appearance of spontaneous seizures, i.e., epilepsy. Although the mechanisms underlying these alterations in the brain are not fully understood, we and others have provided evidence for the involvement of cyclooxygenase-2 (COX-2) cascade in the seizure-triggered pathogenesis. COX-2 is rapidly induced by prolonged seizures and synthesizes prostanoids, which then activate specific G protein-coupled receptors and contribute to brain-blood barrier disruption, brain inflammation and injury, and functional loss. As a dominant product of COX-2 in the CNS, prostaglandin E2 (PGE2) promotes oxidative damage and neurotoxicity in models of chronic inflammation and neurodegeneration, where PGE2 receptor EP2 subtype has been proposed to exert a major role via activating microglia and promoting powerful cytokine storms. Our newly-discovered novel antagonists for EP2 receptor with sufficient pharmacokinetic profiles (Patent: US20140179750) enabled us to directly investigate the candidacy of the EP2 receptor as a therapeutic target to mitigate the pathology following SE. Treatment with these EP2 antagonists significantly reduced glutamate receptor-mediated neuronal excitotoxicity in hippocampal cultures comprising neurons, astrocytes and microglia.
Administration of a brain-permeant EP2 antagonist in mice commencing two hours after seizure onset for two days reduced delayed mortality and functional deficits after a one hour episode of SE induced by kainate or pilocarpine. In addition, EP2 antagonism via this compound also reduced the severity of cytokine storms and neuronal death in hippocampus after SE. In sum, these preclinical findings, together with follow-up studies in other seizure models and species, demonstrate the feasibility of blocking PGE2/EP2 signaling by small molecules to treat SE. The promising salutary actions from these novel EP2 selective antagonists should also be relevant to other neurological conditions including Alzheimer’s disease, multiple sclerosis, strokes, glioblastoma, etc., in which COX-2/PGE2-driven neuroinflammation plays pivotal roles.

Disclosures: J. Jiang: None. R. Dingledine: None.

Poster

494. Epilepsy Mechanisms

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 494.05/F36

Topic: C.07. Epilepsy

Title: Spatiotemporal profile of microglial changes in the hippocampus following prolonged continuous seizure activity in an experimental model of acquired epilepsy

Authors: *S. HERR, N. SCHARTZ, L. MADESN, S. WYATT, J. WOODLIFF, A. BREWSTER;
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Abstract: Long-lasting continuous seizure activity (status epilepticus) (SE) is often associated with hippocampal neuronal and dendritic injury and the subsequent development of spontaneous recurrent seizures (epilepsy). One hallmark often associated with SE-induced hippocampal injury is activation and proliferation of microglial cells, the neuroimmune cells of the brain. Under pathological conditions, microglia infiltrate, accumulate, and develop an inflammatory phenotype that includes a change in morphology from highly ramified to hypertrophied, and the production and release of immunological messenger molecules such as pro-inflammatory cytokines. Also, microglia have been implicated in alterations in dendritic arborization and in synaptic pruning under both physiological and pathological conditions. Thus, we hypothesized that SE-induced activation and proliferation of microglial cells precede the associated hippocampal dendritic pathology. To investigate this possibility we used a combination of immunohistochemistry (IHC) and flow cytometry to map the spatiotemporal profile of SE-induced microglial changes in the hippocampus. SE was induced in rats with the
chemoconvulsant pilocarpine and stopped after one hour with the anticonvulsant diazepam. Controls were given saline. Tissue was fixed at 4 hours, 1-, 3-, 14- and 35-days after SE for IHC and microglia were identified using antibodies against IBA1. Also, hippocampi were dissected at 14 days after SE for flow cytometry analysis using antibodies against CD45, CD11b, and MHC Class II. IHC showed a homogeneous distribution of IBA1 throughout the hippocampus of control rats. SE precipitated microglial morphological changes from ramified to hypertrophied that were evident as early as 4 hrs after SE. Drastic accumulations hypertrophied microglia were evident at 3 days after SE in all hippocampal regions. The remarkable accumulation of microglia in the CA1 area that occurred at 14 days post-SE was resolved by 35 days after SE. Flow cytometric analysis showed an increased number of CD45+/CD11b+/MHCII+ cells 14 days post SE compared to controls. These data support that SE triggers acute changes in microglia activation and promotes a transient proliferation that is very specific to the CA1 area. Furthermore, these data suggest that microglia may contribute to the SE-induced neuronal and dendritic decline that often parallels the time course examined in this study.

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**Poster**

**494. Epilepsy Mechanisms**

**Location:** Hall A

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**Program#/Poster#: 494.06/F37**

**Topic:** C.07. Epilepsy

**Support:** UTEP Faculty Start-up Funds 2G12RR008124

**Title:** The neuroprotective effect of antioxidant compounds in an *in vitro* model of epilepsy

**Authors:** L. P. MONTES, *V. I. NAVARRO, K. FENELON; Biol. Sci., Univ. of Texas At El Paso, El Paso, TX

**Abstract:** Under physiological conditions, radical species such as nitric oxide (NO) are regulatory mediators in various biological processes. However, excess of radical species are also implicated in a wide variety of neurological insults such as stroke and epilepsy. In fact, free radical production is enhanced during epilepsy, leading to seizure-mediated neuronal injury. Experimental seizure-like events are characterized by abnormal synchronized electrical activity and elevated NO seen in various brain regions including the hippocampus. Despite scientific efforts, about 30-40% patients with temporal lobe epilepsy are drug resistant. This is partly
because the current and commonly used anti-epileptic drugs lack anti-oxidant properties to scavenge radical species. Therefore, the development of antioxidant drugs bearing neuroprotective properties should be promising as an additional treatment of epilepsy. Ferrostatin-1 (Fer-1) is a small molecule with antioxidant properties that can prevent cell death (Dixon et al., 2012). Thus here, the objective of the present study was to evaluate the neuroprotective effect of Fer-1 in a simple in vitro model of epilepsy. To do so, we used extracellular field electrophysiological recordings in rodent hippocampal slices. Epileptic form activity was induced in acute hippocampal slices treated with Gabazine (10 µM), a GABAA receptor antagonist, added to the perfusing solution. Gabazine synchronized the activity of excitatory neurons which was shown as repetitive activity in the electrophysiological recordings made in the CA3 region, typical of epileptic-like events. The epileptic activity was quantified by measuring the amplitude and the frequency of the epileptic-like events. Our results show that upon Fer-1 addition, the amplitude of the Gabazine-induced epileptic-like events decreased, whereas their frequency was left unchanged (N = 11). We then decided to test two other Fer-1 analogs (N = 6) also shown to exhibit antioxidant properties assessed through the use of DPPH assay. In addition and as a control, we also compared the effects of Fer-1 to vitamin E, a known antioxidant (N = 2). Among these tested compounds, Fer-1 demonstrated the strongest efficiency by decreasing the amplitude of the epileptic-like events. Fer-1 possibly acted by scavenging the excess NO produced in the hippocampal slice during epileptic activity. We conclude that small antioxidant molecules such as Fer-1 could eventually be combined with currently used drugs as a novel therapeutic strategy for diseases with reactive species imbalances, such as epilepsy.


Poster

494. Epilepsy Mechanisms

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

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Topic: C.07. Epilepsy

Support: NIH Grant NS031718-02A1

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Title: Early life seizures diminish silent synapses in developing cortex
Authors: *H. SUN¹, J. J. LIPPMAN-BELL¹, M. HANDY¹, T. K. HENSCH², F. E. JENSEN¹; ¹Dept. of Neurol., Univ. of Pennsylvania, Philadelphia, PA; ²Dept. of Neurol., Boston Children's Hosp. and Harvard Med. school, Boston, MA

Abstract: Early life seizures are often refractory to conventional antiepileptic drugs and can result in chronic later-life epilepsy and long-term cognitive deficits including autism. Our previous studies have shown that early life seizures increase AMPAR function at least in part due to reduction of NMDAR-only silent synapses in hippocampal pyramidal neurons (Rakhade et al., 2008; Zhou et al., 2011). To determine whether this occurs in other developing synaptic networks, we examined the effects of early life seizures on AMPAR function and silent synapses in developing primary auditory cortex. Early life seizures were induced by daily pentylenetetrazol (PTZ) injections (60mg/kg, i.p.) from P9-11. Whole-cell patch-clamp recordings were performed from Layer IV (L4) A1 pyramidal neurons in auditory thalamocortical slices from P12-13 post-seizure mice and littermate controls. We found that L4 neurons in slices from post-seizure pups showed a significant increase in the amplitude of AMPAR sEPSCs (−13.29 ± 0.64 pA; n = 8; p<0.05) comparable to controls (0.31 ± 0.06 Hz; n = 8). In addition, minimally evoked AMPAR-mediated EPSCs through stimulating single fibers of thalamocortical circuits showed significantly higher amplitudes in neurons from post-seizure mice (18.09 ± 2.21 pA; n = 8, p<0.05) compared to neurons from controls (12.63 ± 1.11 pA; n = 7). We next measured the changes in the ratio of NMDAR-only silent synapses in L4 A1 pyramidal neurons following early life seizures. Silent synapses are determined by the difference of failure rates of evoked eEPSCs at negative membrane potentials (-60 mV) and positive potentials (+40mV). Auditory thalamocortical slices from P12-13 controls exhibited a failure rate of 51.21 ± 4.35% at -60mV and a failure rate of 17.94 ± 2.05% at +40mV (n=8, p<0.05), yielding a significant decrease in the NMDAR-only silent synapses (21.77 ± 6.61%, n=8, p<0.05) compared to controls. Taken together, these data demonstrated the presence of NMDAR-only silent synapses in the developing primary auditory cortex. Importantly, these silent synapses are modifiable and converted to functional synapses by early life seizures, suggesting the potential effects of early life seizures on function and synaptic plasticity of auditory cortex.


Poster

494. Epilepsy Mechanisms

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 494.08/F39
Topic: C.07. Epilepsy

Support: NSERC 222912

Title: The effect of limbic and nonlimbic kindling on hippocampal interneuron populations

Authors: *J. J. BOTTERILL¹, H. J. CARUNCHO², L. E. KALYNCHUK³;
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Abstract: GABAergic inhibitory interneurons are critical in regulating neuronal excitability and cognition. Many factors can alter GABAergic neurotransmission and function within the brain, but one noteworthy factor is epileptic seizures. In the present study we utilized the kindling model of epilepsy in rats to determine whether specific hippocampal GABAergic interneuron populations are affected by seizures. Kindling refers to the gradual development and intensification of elicited motor seizures resulting from electrical stimulation of a discrete brain site. We conducted 99 kindling stimulations of limbic (basolateral amygdala, dorsal hippocampus) and non-limbic (caudate nucleus) brain sites in rats. Within 24 hours of the final kindling stimulation, rats were sacrificed and their brains were prepared for immunohistochemistry. We then conducted profile counts of several GABAergic interneuron markers in the hippocampus, including somatostatin, parvalbumin, calretinin, and GAD67. The results of our study revealed that the number of somatostatin immunoreactive (ir) interneurons were relatively unaffected by kindling. In particular, we found a small increase of somatostatin-ir cells in the CA1 pyramidal cell layer following limbic kindling. However, we also noticed that the hippocampal somatostatin-ir cells of limbic-kindled rats displayed significant hypertrophy (e.g., sprouting), which was confirmed by conducting surface area measurements of somatostatin-ir cells. In contrast, parvalbumin-ir cells was resilient to the effects of kindling and the surface area remained of these cells was also unchanged. We also found that limbic kindling resulted in a very mild reduction of calretinin-ir cells in the dentate gyrus, but no other hippocampal subfields. Lastly, our preliminary results indicate that GAD67 cells are also resilient to the effects of kindling. Taken together, our results suggest that kindling does not cause a gross loss of interneuron populations. Rather, we see small alterations in the absolute number of immunolabeled interneurons, but significant plasticity of specific interneuron populations (e.g., sprouting/hypertrophy of somatostatin-ir cells). Collectively, these results suggest that some interneuron populations undergo greater plasticity than others in response to epileptic insults.


Poster

494. Epilepsy Mechanisms
Enhancing GABA signaling exacerbates febrile seizures and elicits axonal sprouting in the hippocampus

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Febrile seizures (FS) is the most common convulsive event that appears in early childhood. Especially, prolonged complex FSs could be a risk factor for the future development of temporal lobe epilepsy (TLE). To relieve complex FSs, GABA enhancers such as diazepam are widely used for reinforcing the inhibitory action of GABA in neural circuits. However, accumulating evidence suggests that GABAergic signaling depolarizes immature neurons, which has presented questions about the efficacy of GABA agonist for the therapy of neonatal seizures. To evaluate the effects of GABAergic activation during neonatal seizures, we induced complex FSs in mice at postnatal 11 days (P11). Specifically, we investigated the effects of several GABA enhancers including diazepam on the latency to seizure induction, the duration of seizures, and the hippocampal sclerosis, an epileptogenic hallmark suggested as a cause for the future development of epilepsy. We found that mice pre-treated with GABA enhancers demonstrated severer FS phenotypes, such as earlier onset and longer duration of seizures compared to control FS mice without the treatment of GABA enhancers. Among several GABA enhancers, the effect of pentobarbital on the seizure phenotype was the most prominent. Then we further determined the long-term effects of pentobarbital on FSs by analyzing anatomical changes in the hippocampus at P60 such as the dispersion of dentate granule cells, the hippocampal mossy fiber sprouting, and the degeneration of CA3 pyramidal cells, all indicative of the hippocampal sclerosis. The dispersion of dentate granule cells was comparable between control, FS, and FS + pentobarbital mice. In contrast, the mossy fiber sprouting was observed only in FS + pentobarbital mice. In addition, the number of unhealthy cells with aggregating DAPI staining in CA3 was increased in FS mice compared to control, which was prevented in FS + pentobarbital mice. In conclusion, our findings suggest that GABA enhancers could exaggerate early-life FSs.
febrile seizure in immature brain and cause the mossy fiber sprouting, while they may prevent FS-induced cell loss in CA3.

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Poster

494. Epilepsy Mechanisms

Location: Hall A

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Topic: C.07. Epilepsy

Title: Comparing microarray profiles of hippocampal subregions with amygdala cortical complex reveals distinct gene expression following multiple early life seizures

Authors: *L. K. FRIEDMAN*1,2, S. HU1, A. M. SLOMKO1, K. C. YEE1, J. M. MANCUSO2; 1New York Med. Col., Valhalla, NY; 2Neurosci., NYIT, Old Westbury, NY

Abstract: Previously we showed that postnatal (P) P20 rats are relatively resistant to hippocampal CA1 injury when they have a history of two earlier neonatal seizures, whereas other limbic areas are less spared. Transcriptome profiling of the CA1 subregion following single (1×KA) and multiple early life seizures (3×KA) showed many common and uncommon genes were up and downregulated. To gain further understanding of which genes are actually involved in spatially protective vs. neurotoxic effects, we profiled genes of the CA3, dentate gyrus (DG), and amygdala entorhinal complex (Amg/Ercx) under similar conditions. Within the CA1 and CA3, autophagy and pro-inflammatory genes were triggered; however, many protective genes were also differentially upregulated, particularly after 3×KA. These included but were not limited to Ca++ modulated proteins, apoptosis inhibitors, adaptor-related protein complexes, ADAM metalloproteinases, adaptor ATG autophagy genes, caspase cascade activators, ATP-mediated gliotransmitters, GTP binding proteins, cyclins, F-box proteins, growth factors, interleukins, heat shock proteins, certain GABAergic, ionotropic and metabotropic glutamate neurotransmitter receptors and synthesizing enzymes. Differential downregulation included genes encoding ankyrin-repeat proteins, adenosine receptors, ATP synthases, caspases, Ca++ channels, certain heat shock proteins, NFKB activating proteins, synaptosomal associated proteins, K+ voltage gated channels, and zinc finger domains. In contrast, within the DG, autophagy, pro-inflammatory, pro-apoptotic, and anti-apoptotic transcripts were absent. Instead, dual specificity phosphatases that negatively regulate members of the mitogen-activated protein (MAP) kinase superfamily, axonal and vesicular motility (e.g. dynein) and ATP/ITP metabolism gene regulators predominated which likely contribute to increased Ca++ transport.
Downregulated genes of the DG included ankyrins, reelin, vimentin and other adhesive and guiding transcripts, ionotropic GluR1 subunit, and several G-protein-coupled receptors. In contrast, the Amg/Ercx showed unique upregulation of cholinergic nicotinic and adrenergic receptors, and distinguished downregulation of dopamine, cholinergic muscarinic, histamine, and serotonergic receptors. Results indicate that sustained seizures during early life induce marked region specific brain differences in a large number of critical neurotransmitter genes that encode glutamatergic, adrenergic, cholinergic, and monomeric receptors to influence the seizure threshold, neuronal vulnerability, proliferation and migratory domains.


Poster

494. Epilepsy Mechanisms

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 494.11/F42

Topic: C.07. Epilepsy

Title: Alterations in long-term potentiation (LTP) and gene expression of ionotrophic glutamate receptors and neurotrophic factor caused by undernourishment, recurrent neonatal seizures and environmental enrichment


Abstract: Undernourishment associated with recurrent neonatal seizures induces the reduction in brain cell number and suppression of glutamate synapses, causing deficits in spatial learning and memory. It has been shown that environmental enrichment improves learning and retention of memory and enhances long-term potentiation in the hippocampus, being able to reverse the cognitive impairment generated by undernourishment and recurrent seizures. The purpose of the present study was to evaluate the alterations of undernourishment, recurrent neonatal seizures and environmental enrichment in LTP and the expression of genes related to neuroreceptors and neurotrophins. Male Wistar rats were divided in two groups: undernourished + seizures (US) and nourished (N). Undernourishment model consisted in maternal and nutritional deprivation from postnatal day 2 (P2) to P15. From P7 to P10, recurrent seizures were induced in the rats by fluorothyl exposition five times per day. Both groups were exposed to the enriched environment between P30 and P60. At P61, the animals were euthanized and one hippocampus was separated
for in vitro electrophysiological study (LTP) and the other one for evaluation of expression of NMDA receptor subunits (NR1A, NR2A, NR2B, NR2C and NR2D), AMPA receptor subunits (GluR1, GluR2 and GluR3) and BDNF by qRT-PCR. Preliminary data demonstrated a hippocampal LTP induction in nourished group after environmental enrichment, unlike the undernourished + seizures group in which no induction of LTP was obtained, despite being exposed to the environment enriched. There were an increase in the NR1A, NR2D, GluR2 and BDNF expression genes and a decrease in the NR2A, NR2B, NR2C, GluR1 and GluR3 expression genes in the hippocampus in all groups.


Poster

494. Epilepsy Mechanisms

Location: Hall A

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Support: UFABC

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Title: Effects of the intrahippocampal injection of dantrolene in the expression of synaptic plasticity-related proteins during epileptogenesis

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Abstract: Status epilepticus (SE) is a clinical emergency that can lead to the development of temporal lobe epilepsy (TLE) after neuronal injury. The term epileptogenesis refers to the transformation of the normal neuronal network into a long lasting chronically hyperexcitable state. In most patients presenting TLE, the development and maintenance of spontaneous seizures are linked with hippocampus scleroses-induction, which comprise neuronal loss, reactive gliosis and synaptic modifications following the first injury. It has been shown that SE
produces increase in ryanodine-dependent intracellular calcium levels in hippocampal neurons that remain elevated in animals that develop epilepsy. The aim of this work was to investigate the participation of intracellular calcium released from ryanodine receptors during epileptogenesis, by analyzing the pattern of distribution of some synaptic plasticity-related proteins such as PSD-95, synapsin I and the activity-regulated cytoskeleton-associated protein (arc). Male wistar rats weighting 290-320 g were submitted to stereotaxic surgery for cannula implantation in CA1 region of the hippocampus. After a recovery period of ten days, animals were treated with methyl-scopolamine (1 mg/kg, subcutaneous) followed by pilocarpine injection (360 mg/kg, intraperitoneal). Thirty minutes after the establishment of SE, the ryanodine receptor blocker dantrolene (1 mM) was administered through intrahippocampal injection. Control animals received saline diluted in vehicle instead of dantrolene. A third group consisted of animals that were not submitted to pilocarpine injection, named sham group. One hour after dantrolene or saline treatment, animals received diazepam (10 mg/kg, subcutaneous) in order to interrupt the SE. Animals were sacrificed 48 hours after SE induction and the brains were collected for immunofluorescence analysis. Our results showed that pilocarpine-induced SE caused increased immunolabeling of synapsin I and PSD-95 principally in CA1 region, while arc immunoreactivity was more intense in all regions of the hippocampus. Dantrolene treatment did not affect the expression of the synaptic proteins synapsin I and PSD-95. However, dantrolene decreased the number of cells highly immunoreactive for arc, especially in the CA3a subregion, producing a pattern of staining similar to the sham group in this region. Our results suggest that the dynamic changes in intracellular Ca2+ concentration produced by ryanodine receptors may play a role in the structural changes produced after SE that could lead to the development of epilepsy.

Authors: *L. G. MEDINA-CEJA, C. R. SÁNCHEZ-CASTAÑEDA, X. N. FLORES-PONCE, A. MORALES-VILLAGRÁN; Univ. de Guadalajara, Zapopan, Mexico

Abstract: *In vitro* studies in the rat hippocampus (HIP) have found epileptiform activity in the absence of chemical synapses. This fact suggests that other mechanisms may be involved in epilepsy, such as electrotonic coupling between cells. This electrotonic coupling is produced by gap junctions (GJs). GJs are formed by the combination of two hemichannels, each composed of six connexins. The convulsive drug 4-aminopyridine (4-AP) produces epileptiform activity even at low doses, without affecting glutamate levels; therefore, GJs could participate in its effect. Accordingly, in this study, the expression of Cx 32, Cx 36 and Cx 43 protein and mRNA in the HIP of rats treated with 4-AP was evaluated. The evaluation of connexins was carried out by chemifluorescent immunoassay, semiquantitative RT-PCR and immunofluorescence to detect the amount and distribution of connexins and their cellular markers in the HIP and dentate gyrus (DG) of animals treated with NaCl and 4-AP in the right entorhinal cortex. Also, in these animals, convulsive behavior and EEG signals were analyzed. The 4-AP treated animals showed convulsive behavior and epileptiform activity 60 min after the administration. A significant increase in the protein expression of Cx 32, Cx 36 and Cx 43 was found in the HIP contralateral and ipsilateral to the site of 4-AP administration. A trend toward an increase in the mRNA of Cx 32 and Cx 43 was also found. An increase in the cellular density of Cx 32 and Cx 43 was found in the right HIP and DG, and an increase in the cellular density of oligodendrocytes in the DG and a decrease in the number of cells marked with NeuN were observed in the left HIP. In conclusion the Cx 32 and Cx 43 associated with oligodendrocytes and astrocytes, respectively have an important role in the first stages of seizures induced by 4-AP, whereas Cx36 localized in neurons could be associated with later stages. In addition, these results contribute to our understanding of the role of connexins in acute seizures and the possibility about other new anticonvulsant strategies for seizure treatment. Support contributed by the grant from LMC, CONACYT-SEP-CB 106179.

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Poster

494. Epilepsy Mechanisms

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Topic: C.07. Epilepsy
Prolonged seizures trigger transient alterations in NeuN and Map2 expression in hippocampal CA1 cells

Authors: N. D. SCHARTZ, L. MADSEN, R. MURILLO, *A. L. BREWSTER; Psychological Sci., Purdue Univ., West Lafayette, IN

Abstract: Epilepsy is characterized by spontaneous recurrent seizures (SRS) that are often drug resistant. The complexity of epilepsy is broadened by brain injury associated with episodes of prolonged continuous seizures (status epilepticus) (SE). SE can severely disrupt vulnerable neuronal networks such as those in the hippocampus thereby increasing the predisposition for SRS, the development of temporal lobe epilepsy (TLE), and cognitive comorbidities. Several studies in well-established models of SE and acquired TLE support that single episodes of SE promote long-lasting homeostatic instability of hippocampal neurons and dendrites that may contribute to neuronal hyperexcitability and aberrant synaptic plasticity. Some of the associated events include reduction or loss of the dendritic protein Microtubule associated protein 2 (Map2) and the neuronal marker NeuN. However, the time course of SE-induced changes in these proteins has yet to be described. Therefore, in this study we determined the temporal pattern of SE-induced changes in Map2 and NeuN in the hippocampal formation. We used immunohistochemistry (IHC) with antibodies against NeuN and Map2 to stain neurons and dendrites, respectively. Pilocarpine was used to induce 1 hr of SE which was stopped with diazepam. Rats were then perfused at various time points after SE (4 hr, 1-, 3-, 7-, 14-, and 35-days). IHC showed strong NeuN staining within the hippocampal CA1-3 pyramidal and granule cell layers and dense Map2 signal throughout all the dendritic regions in control hippocampi. After a single episode of SE a transient decline in NeuN and Map2 immunostaining was evident mainly within the CA1 region between 3-14 days after SE. Interestingly, at 3 days post SE the Map2 signal was reduced from the CA1 stratum radiatum and was more intense within the CA1 pyramidal cell body layer compared to controls. At 2 weeks, Map2 staining was drastically reduced from both cell bodies and dendritic fields of CA1 cells relative to control hippocampi. Despite the drastic loss of Map2 at 2 weeks post-SE, golgi staining at this time point showed vast dendritic arborizations in CA1 in morphologically viable cells. These SE-induced changes in NeuN and Map2 were not evident 5 weeks post-SE. Instead, at 5 weeks NeuN and Map2 signal were more robust that at 2 weeks post SE and just slightly weaker than controls suggesting a transient decline in the expression of these markers. Taken together these data suggest that SE promotes a transient decline NeuN and Map2 proteins that may be associated with a homeostatic instability of viable hippocampal cells.


Poster

494. Epilepsy Mechanisms
Location: Hall A
Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM
Program#/Poster#: 494.15/G2
Topic: C.07. Epilepsy
Support: NIH NS35439

Hewitt Foundation Biomedical Research

Title: The complex role of microRNA-124 in epileptogenesis

Authors: *G. P. BRENNAN¹, D. DEY¹, K. P. PATTERSON², E. J. MAGNETTA¹, A. HALL¹, Y. MEI¹, T. Z. BARAM¹;
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Abstract: Introduction: Epileptogenic insults such as status epilepticus (SE) induce rapid changes in cellular properties caused by large scale changes in gene expression and regulation. Among the regulatory pathways triggered by epilepsy-inducing insults and might contribute to epileptogenesis, are the transcriptional repressor NRSF and the inflammatory cascade. Inhibiting NRSF activity following SE significantly ameliorated, but did not eliminate, the subsequent epilepsy (1). Efforts to block inflammation have also failed to prevent epilepsy (2) suggesting that targeting of single pathways may be insufficient to alter disease course. Therefore, identification of key molecules which regulate multiple epileptogenic pathways could lead to the development of effective anti-epileptogenic therapies. MicroRNAs are small non coding RNAs which post-transcriptionally regulate gene expression by targeting mRNA. Recent work suggested that mir-124 acts as a repressor of inflammation, and has been reported to regulate NRSF during neuronal maturation. Here we tested if mir-124 might play a dual role in epileptogenesis by regulating both NRSF and inflammation and investigated its therapeutic potential. Methods: SE was induced in adult rats by systemic KA. mRNA levels were quantified by qPCR. Protein levels were measured by w.blot. To test the effect of miR-124 treatment on epileptogenesis rats received miR-124 mimic infusion via ICV after SE and were monitored using 24h video-EEG for 60 days. To determine miR-124 mediated regulation of NRSF expression and inflammatory pathways, miR-124 mimics were infused via ICV, immediately after SE cessation. Results: SE robustly reduced hippocampal mir-124, and increased NRSF mRNA and protein levels as well as many pro-inflammatory cytokines. MiR-124 restitution failed to prevent epilepsy development. We explored why miR-124 had no effect on seizure development. MiR-124 restitution prevented seizure-induced NRSF expression and activity however, unexpectedly, miR-124 mimics exacerbated inflammation, indicating a dual counter-balancing role of miR-124 in epileptogenesis. Conclusion: MiR-124 plays a complex role in epileptogenesis. Reduced miR-124 is pro-epileptogenic leading to increased NRSF expression
and activity. While reduced miR-124 is anti-epileptogenic because reduced miR-124 dampens the inflammatory response induced by epileptogenic insults. Although miR-124 restitution was unable to prevent epileptogenesis we have identified the regulatory mechanisms underlying aberrant NRSF activity during epileptogenesis and also revealed a more complex role for miR-124 in regulating inflammation.


Poster

494. Epilepsy Mechanisms

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 494.16/G3

Topic: C.07. Epilepsy

Support: CIHR

Title: Visualization of post-seizure vessel constriction in acute hippocampal slices

Authors: *L. S. DAVID*<sup>1</sup>, J. S. FARRELL<sup>2</sup>, G. C. TESKEY<sup>3</sup>;
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Abstract: Our laboratory has demonstrated in adult rats that following seizure termination, the areas of the brain involved in the seizure are subjected to a prolonged period of severe hypoxia (pO2 < 10 mm Hg) that often lasts over an hour. We also showed that this hypoxia was in part mediated by a reduction in blood flow as we observed a ~30% decrease in perfusion using an indirect measure; laser Doppler flowmetry. This was supportive evidence of a long-lasting vasoconstriction, but we wanted to directly measure a change in vessel diameter in relation to post-seizure hypoxia. The first goal of this study was to examine the characteristics of post-seizure hypoxia <sup>in vivo</sup> using young rats, since young rats are better suited for work in acute hippocampal slices. Using both standard electrical kindling and 3 Hz stimulation on P25 to P40 rats <sup>in vivo</sup>, we observed hippocampal pO2 levels fall below 10 mmHg and lasting approximately an hour, much like adults rats. In order to determine if the seizure-induced severe hypoxic episode could result in vasoconstriction, we investigated blood vessel diameter using transverse hippocampal slices following 2 minutes of 3 Hz stimulation applied to Schaffer collaterals in rat hippocampus. We measured lumen diameter before and after stimulation to

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determine the amount of vasoconstriction. We also determined if we could prevent vasoconstriction both in vivo and in vitro using the nonspecific COX-2 antagonist acetaminophen. The results from this study potentially provide insights into the development of preventative therapeutic approaches to prevent ischemia/hypoxia induced by seizures.


Poster

494. Epilepsy Mechanisms

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 494.17/G4

Topic: C.07. Epilepsy

Support: CIHR

NSERC

Title: Does the ischemia/hypoxia that follows seizures contribute to brain damage?

Authors: *J. S. Farrell*,1,3 G. C. Teskey1,3;

1Neurosci., 2Cell Biol. and Anat., Univ. of Calgary, Calgary, AB, Canada; 3Hotchkiss Brain Inst., Calgary, AB, Canada

Abstract: Seizures can result in a pathology that is remarkably similar to that seen following stroke. Neuronal death, glial activation, blood-brain barrier permeability are common features of both insults suggesting a common link. Using implantable oxygen sensors, laser Doppler flowmetry, and immunohistochemistry, we previously demonstrated that following seizure termination, local tissue oxygenation drops to severely hypoxic levels (<10mmHg) which co-occurs with a ~30% drop in blood flow. This ischemic/hypoxic period lasts for over an hour and is specific to brain regions involved in the seizure. Furthermore, this phenomenon is prevented by pre-administration of COX-2 antagonists. Using COX-2 antagonists as a tool to dissociate the seizure from the ischemic/hypoxic event, we hypothesized that it is the ischemic/hypoxic event following seizures (and not the seizure itself) that causes pathological changes to the brain. We approached this hypothesis by examining anatomical changes following 5 seizures elicited over 3 days. We expect that the acute effects from the most recent seizure (on day 3) and enduring changes from previous seizures (days 1 and 2) should capture a complex range of damage. We examined microglia and astrocyte activation, markers of neuronal death, and release of HMGB1 in rats that experienced seizures with hypoxia (vehicle) or without hypoxia (acetaminophen). In a
separate experiment, to assess blood-brain barrier permeability, we examined the leakage of circulating Evan’s Blue dye into the brain parenchyma 45-minutes following seizures with or without hypoxia. Given the potent effects of ischemia/hypoxia on the indices of damage under examination, we expect that the hypoxia observed after seizures plays a major role in the detrimental effects of seizures. These experiments are important because if therapies (COX inhibitors) are highly effective in preventing damage induced by this phenomenon, they could serve as candidates for translation to the clinic.

Disclosures: J.S. Farrell: None. G.C. Teskey: None.

Poster

494. Epilepsy Mechanisms

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

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Topic: C.07. Epilepsy

Support: CIHR

Title: Long-term amelioration of seizure-induced hypoxia: Effect on epileptogenesis and behavioural disturbances

Authors: *M. D. WOLFF, S. C. SPANSWICK, J. S. FARRELL, G. C. TESKEY;
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Abstract: We recently determined that following cessation of brief seizures, a long-lasting, severe hypoxic event occurs in the brain regions involved in the seizure. Following a hippocampal seizure, for example, local tissue oxygenation drops below 10 mmHg and remains below this severe hypoxic level for over an hour. Previous research has demonstrated that repeated hippocampal seizures in rats results in behavioural deficits in hippocampal-dependent memory tasks. However, the contribution of the hypoxic period that follows a seizure on these tasks has not been determined. We hypothesized that rats which received 20 electrically kindled seizures but without severe hypoxia (via pre-administration of acetaminophen) will not have deficits in hippocampal-dependent memory tasks relative to rats that have seizures and post-seizure hypoxia. We also hypothesized that prevention of seizure-induced hypoxia will modulate the epileptogenic process (epileptogenesis). We used the electrical kindling model to induce brief seizures. Adult male Long-Evans rats were implanted with a chronic bipolar electrode in the ventral hippocampus and a pO2 detecting optrode into the dorsal hippocampus. Oxygen levels in the hippocampus were measured with a platinum-based fiber-optic probe, which provided a
recording of local pO2 levels in an awake, freely moving rat. We separated rats into five groups:
(1) no seizure; (2) no seizure + vehicle; (3) no seizure + acetaminophen; (4) seizure + vehicle;
(5) seizure + acetaminophen. Thirty minutes prior to each kindling or sham kindling session, rats
were injected (i.p.) with either vehicle or acetaminophen. Seizures were elicited once per day
until 20 seizure-induced severe hypoxic events occurred in the seizure + vehicle group. Twenty-
four hours following the final kindling session, behavioural testing was initiated. Behavioural
testing consisted of novel object/context mismatch and a moving platform version of the Morris
water task. To analyze the contribution of seizure-induced hypoxia on epileptogenesis, we
measured the afterdischarge duration and behavioural stage of each seizure.

Disclosures: M.D. Wolff: None. S.C. Spanswick: None. J.S. Farrell: None. G.C. Teskey:
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Poster
494. Epilepsy Mechanisms

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Topic: C.07. Epilepsy

Support: NHMRC 1044407

Fondesct Initiations into Research Grant 11130232

Title: Glucose metabolism is unchanged despite impairments in TCA cycling in the chronic
epileptic stage of the pilocarpine model

Authors: *T. MCDONALD*1,2, M. HODSON3, C. CARRASCO-POZO2,4, K. BORGES2;
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Queensland, St Lucia, Australia; 4Dept. of Nutr., The Univ. of Chile, Santiago, Chile

Abstract: There is growing evidence that dysfunctions in energy metabolism play a role in the
pathophysiology of epilepsy with reports that amino acids produced from glucose metabolism
such as glutamate and glutamine are reduced. However little is known about where the
perturbations in the metabolic pathways of glucose occur. Here we used assessment of the percent
enrichment of carbon-13 in various metabolites extracted from hippocampi following the
injection of U-13C glucose (i.p.) during the chronic phase of the pilocarpine status epilepticus
(SE) model of epilepsy to identify the problem in metabolism. The carbon-13 enrichment in
hippocampal intermediates of the glycolytic, pentose phosphate and TCA cycle intermediates
were measured via liquid chromatography tandem mass spectroscopy. No changes in the enrichment of carbon-13 was found in either glycolytic or pentose phosphate pathway intermediates. These data correlate with the lack of significant changes in the maximal activity of enzymes involved in these pathways, thus indicating that hippocampal glucose utilisation is not altered in the chronic epileptic stage. The percent enrichment of carbon-13 resulting from the first turn of the TCA cycle was reduced in citrate (17%**), aconitate (17%*), succinate (35%**), fumarate (20%**) and malate (20%*). This reduction was even more pronounced in the percent enrichment in citrate (34%), α-ketoglutarate (47%*) and succinate (54%**, all n=10-11), produced in the second turn of the carbon-13 label in the TCA cycle, indicating a reduction in TCA cycling, which is further corroborated with a 55%* (n=7-8) loss in the maximal activity of α-ketoglutarate dehydrogenase. We are currently assessing if pyruvate dehydrogenase activity and/or oxidative phosphorylation are impaired. Together our results indicates that the impairments in the TCA cycle are not due to changes in glycolysis. The lack of enrichment in the TCA cycle intermediates may be due to reduced pyruvate entry, however the further reduction in enrichment during the second cycle indicates there is reduced TCA cycling during the chronic phase and thus impaired NADH production. Taken together, we show dysfunctions in energy metabolism in the epileptic brain, which support the use of alternative fuels that feed into the TCA cycle to improve cycling and thus energy production.

Disclosures: T. McDonald: None. M. Hodson: None. C. Carrasco-Pozo: None. K. Borges: None.

Poster

494. Epilepsy Mechanisms

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Topic: C.07. Epilepsy

Support: Funding was provided by Biomedical Advanced Research and Development Authority (BARDA) via an interagency agreement with the USAMRICD

Title: Neuropathology and morphological changes in dendritic spines caused by sarin-induced seizures in juvenile female rats

Authors: *F. ROSSETTI¹, L. K. WRIGHT², L. A. LANGE²;
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Abstract: Chemical warfare nerve agents (CWNA), such as sarin (isopropyl methyl phosphonofluoridate, GB), produce status epilepticus, extensive neuropathology and long-term performance deficits if seizures are not controlled. Seizures, as caused by CWNA, may directly affect the morphological and functional properties of dendritic spines, and these changes may lead to the cognitive deficits associated with epilepsy. We investigated the effects of GB-induced seizures on dendritic spines of the CA1 region of the hippocampus and the basolateral amygdala (BLA) of juvenile female rats. Female Sprague-Dawley rats were exposed subcutaneously to GB (0.6 or 1.0 LD50) or saline on postnatal day (PND) 42. The rats were deeply anesthetized at 1, 6, 24, or 72 h post-exposure, and brains were removed and fixed in Golgi-staining solution from FD Neurotechnologies, Inc. Dendritic spines were quantified by Sinq Systems, Inc., to include spine density, lengths of dendrites and branches in hippocampal CA1 and BLA. Spectral analysis of EEG was analyzed to calculate the time spent in seizures (TSS). The EEG TSS analyses showed that only 1.0 LD50 GB caused seizures as seen at the 1 h (966 ± 391 s), 6 h (7617 ± 1861 s), 12 h (12395 ± 5503 s), and 72 h (18780 ± 8227 s) time points. These rats showed reduction of dendritic spine density in BLA (0.896 ± 0.026 spines/µm2) in relation to the saline group (1.101 ± 0.022 spines/µm2) 6 h after exposure. Analysis of the number of branches per Golgi+ neurons and length of the dendrites showed that, 24 h after 1.0 LD50 GB, the CA1 had shorter dendrites per neuron (1.29 ± 0.09 mm) in relation to the saline group (1.66 ± 0.15 mm). The BLA showed a decreased number of branches per neuron (13.01 ± 1.13 and 12.57 ± 1.79, 1 h, and 72 h respectively), and shorter dendrites per neuron (0.45± 0.04 mm and 0.45 ± 0.06 mm, 1 h and 72 h respectively) after 1.0 LD50 GB exposure, in relation to saline group: branches per neuron (19.17 ± 1.19 and 19.07 ± 1.33, 1 h and 72 h respectively); length per neuron (0.64 ± 0.03 mm and 0.65 ± 0.04 mm, 1 h and 72 h respectively); p<0.05, ANOVA test. The seizures induced by exposure to 1.0 LD50 GB led to a reduction in the length of dendritic spines in CA1 and BLA neurons, as well as a reduction in the number of branches and spine density in BLA neurons of PND 42 female rats. These results show a strong deficit in dendritic growth in the CA1 and BLA and the sensitivity of BLA dendritic spines when these females were exposed to a seizure-inducing dose of GB (1.0 LD50). In conclusion, seizures induced by exposure to 1.0 LD50 GB cause severe formation changes in the dendritic spines in juvenile female rats, mainly in the amygdala formation, which may lead to significant cognitive deficits in adulthood.

Disclosures: F. Rossetti: None. L.K. Wright: None. L.A. Lange: None.

Poster

494. Epilepsy Mechanisms

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 494.21/G8
Title: Developmental plasticity of dentate gyrus granule cells following epileptiform activity in vitro

Authors: *K. P. PATTERSON*, Y. CHEN, Y. NOAM, G. P. BRENNAN, C. LY, T. Z. BARAM;
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Abstract: Introduction: The dentate gyrus of the hippocampus is among the few regions of the brain where neurogenesis continues throughout life. Epilepsy inducing insults such as status epilepticus alter both the rate of neurogenesis and the developmental outcome of newly generated or immature dentate gyrus granule cells (DGCs). Normally, immature DGCs have a basilar dendrite that is then lost, so that mature DGCs have only apical dendrites that fan into the DG molecular layer. Weeks after epilepsy inducing insults, dysmature DGCs with basilar dendrites are found, presumably representing aberrant stabilization of the basilar dendrites by network hyperactivity. Basilar dendrites may be important for epilepsy because they can create hyper-excitability loops within the dentate gyrus making for a seizure-prone hippocampal circuit. Understanding the mechanisms by which basilar dendrites are retained, leading to aberrant connectivity, may lead to interventions that abort epileptogenesis. Methods: Hippocampal organotypic slice cultures were obtained from P7 Thy1-YFP mice. Seizure-like events were induced at DIV 7 by incubating the cultures for 24 hours in a medium containing kainic acid (6 µM; KA). Controls were exposed to normal medium. BrdU was applied to the cultures at various timepoints before or after the KA. At DIV 21 and 28, cultures were fixed with fresh, ice cold 4% PFA. Cultures were washed with 0.01M PBS, mounted on glass slides and coverslipped. Endogenous YFP in DGCs were visualized using fluorescent microscopy. All assessments were conducted blinded. Measured parameters include: the numbers of YFP positive DGC per culture and the proportion of these cells that maintain basal dendrites. Results: Somata and dendrites of DGCs were clearly filled with YFP. The introduction of KA to culture medium had a significant effect on the number of DGC: There were more YFP + DGCs in cultures with KA than in controls (T= 225.0, p= 0.0027; Mann-Whitney’s U test). Furthermore, there was a significant increase in both the number of DGCs with basilar dendrites in the epileptiform group at both time points (e.g., DIV 21: p= 0.0149) and the ratio of total YFP positive cells over DGCs with basilar dendrites e.g., (DIV 21: p=0.0356). The birth-dates of the dysmature cells are under evaluation. Discussion: 1. KA in vitro causes increased DGC birth, providing a useful system to study changes in DGCs following epilepsy provoking insults. 2) Using this system, an increase in basilar dendrites on DGCs is found mimicking in vivo models. 3) Mechanisms of DGC dysmaturation may now be studied using this system, focusing specifically on the potential role of augmented NRSF after epilepsy provoking insults.

Poster

494. Epilepsy Mechanisms

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#/Poster#: 494.22/G9

Topic: C.07. Epilepsy

Support: FIRB 2010 RBFR10ZBYZ_003

Title: Visual processing in a mouse model of focal neocortical epilepsy

Authors: A. PANARESE1,2, A. MAZZONI2, E. VANNINI1, M. PIETRASANTA1, S. LAI2, S. MICERA2,3, M. CALEO1, *L. RESTANI1;
1CNR Neurosci. Inst., Pisa, Italy; 2The BioRobotics Institute, Scuola Superiore Sant’Anna, Pisa, Italy; 3Ecole Polytechnique Federale de Lausanne, Lausanne, Switzerland

Abstract: Epilepsy is the second most common neurological disorder after stroke, affecting over 0.5% of the world population. A percentage of patients (30%) remain resistant to drug treatments. This pharmacoresistance predominantly affects patients with focal epilepsy, which are 40-50% of total incidence cases. Electrophysiological studies of refractory epilepsy are currently in progress to get insights into the mechanisms of circuit remodeling, thus paving the way for alternative therapeutic options. However, how plastic rearrangements within the epileptic focus trigger cortical dysfunction and hyperexcitability is still incompletely understood. We employ a model of neocortical, non-lesional epilepsy based on local delivery of the clostridial enzyme tetanus neurotoxin (TeNT) in mouse visual cortex. TeNT is a metalloprotease that enters synaptic terminals and cleaves the synaptic vesicle protein VAMP/synaptobrevin, resulting in preferential blockade of inhibitory neurotransmission. Delivery of TeNT to the adult cortex results in refractory epilepsy with electrographic seizures persisting for several months, even after the toxin has been cleared from the system (Mainardi, Pietrasanta et al. 2012). Here we use anesthetized TeNT mice to investigate how epileptic rearrangements impact on sensory processing. We recorded local field potentials and spiking activity both during baseline conditions and after presentation of visual stimuli, in control and TeNT-injected mice, at the completion of TeNT effects (i.e 45 days following injection). In TeNT-treated cortices we found that spontaneous neuronal discharge was increased and visual responses were less reliable, with a higher proportion of failure trials, associated to higher spiking activity before stimulus presentation. Electrophysiological and behavioral visual acuity (spatial resolution) was also
consistently impaired in TeNT-injected mice. We also investigated how contrast-driven modulations in spiking activity and local field potential spectra were affected by TeNT injection. These analyses will shed light on circuit modifications associated to epileptic activity. To further understand layer-specific rearrangements within the epileptic focus, we recorded control and TeNT mice using multichannel linear probes (16 channels), spanning the whole cortical thickness, allowing a layer-specific analysis. Our data contribute to elucidate neuronal mechanisms underlying the network hyperexcitability observed in neocortical focal epilepsy.

**Disclosures:** A. Panarese: None. A. Mazzoni: None. E. Vannini: None. M. Pietrasanta: None. S. Lai: None. S. Micera: None. M. Caleo: None. L. Restani: None.

**Poster 494. Epilepsy Mechanisms**

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**Topic:** C.07. Epilepsy

**Support:** Natural Science Foundation of Jiangsu Province (BK20141335 to Xinjian Zhu)

Specialized Research Fund for the Doctoral Program of Higher Education (20130092120043 to Xinjian Zhu)

**Title:** Neuronal nitric oxide synthase contributes to pentylenetetrazole-kindling-induced hippocampal neurogenesis

**Authors:** *X. ZHU;
Med. Sch. of Southeast Univ., Jiangsu, China

**Abstract:** Neuronal nitric oxide synthase (nNOS), the major nitric oxide synthase isoform in the mammalian brain, is implicated in the pathophysiology of several neurological conditions, including epilepsy. Seizure induced neurogenesis in hippocampal dentate gyrus (DG) have been widely accepted to be associated with epileptogenesis. Few studies, however, have addressed the role of nNOS in seizure associated neurogenesis. The present study, therefore, investigated the role of nNOS in pentylenetetrazole (PTZ)-induced kindling as well as PTZ-kindling-induced neurogenesis in hippocampal DG. Our results showed that nNOS expression and enzymatic activity were significantly increased in the hippocampus of PTZ-kindled mice. Meanwhile, these PTZ-kindled mice were characterized by significant enhancement of new born cells proliferation and survival in hippocampal DG, and these survived cells are mostly co-labeled with NeuN and
only a small portion were co-labeled with GFAP, indicating a vast majority of kindling-induced new born cells differentiated into neurons in hippocampal DG. Selective inhibition of nNOS by 7-NI, however, suppressed PTZ-induced kindling as well as PTZ-kindling-induced hippocampal DG new born cells proliferation and survival, suggesting that nNOS contributes to PTZ-kindling-induced hippocampal neurogenesis and this PTZ-kindling-induced neurogenesis might be associated with kindling development.

Disclosures: X. Zhu: None.

Poster

494. Epilepsy Mechanisms

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Topic: C.07. Epilepsy
Support: CONACYT-SEP-CB 106179
Title: Serotonin receptor antagonists increase fast ripple activity in rats treated with kainic acid
Authors: *C. G. GARCÍA-BARBA, L. MEDINA-CEJA;
Ctr. Universitario De Ciencias Biológicas Y Agro, Zapopan, Mexico

Abstract: Fast ripples (FR) are pathological high frequency oscillations (250-600Hz) associated with epileptic activity in the hippocampus and are an important biomarker in temporal lobe epilepsy as well as in extratemporal epilepsies. Successful modulation of FR by serotonin was observed in a previous study of this laboratory, in which the elevation of serotonin levels induced by citalopram (4.14 ± 0.30 nM) reduced the occurrence of spontaneous FR (57%), the mean number of oscillation cycles per FR event (34%) and the average frequency of FR (33%). Accordingly, we assumed that inhibition of serotonin by the receptor antagonists increase FR in rats treated with kainic acid (KA). For this propose, intrahippocampal KA treated animals (dose 0.8 µg/0.5 µl) of fifteen days post administration, were implanted with a mobile device with eight microelectrodes into the right region of the hippocampus in order to easily detect FR. Control implanted rats were injected with WAY 100135 (dose 0.2 mg/kg, i.p.; n=3), Ritanserin (dose 0.2 mg/kg, i.p.; n=3) and only KA (0.8 µg/0.5µl). For the experimental group (n=6) the first antagonist (WAY 100135) was injected 24 hours after the surgery and the second antagonist (Ritanserin), 72 hours after the first injection. The evaluation of the frequency of appearance of spontaneous FR, number of oscillations per event of FR, frequency and duration of each event of FR were analyzed. Results showed that animals from control and experimental
groups had normal behavior and none of the experimental animals presented seizures during the EEG recordings. The intracranial EEG analysis showed slow activity in control groups and fast activity within the range of ripples was observed particularly during sleep. WAY 100135 increased the number of events of FR significantly during the first 60 minutes after the administration of the antagonist (p<0.0001), and there was an increment in the duration of each FR event during 120 minutes after the administration of the drug (p<0.030), while Ritanserin increased the number of events of FR within the first 60 minutes after its administration (p<0.0001), and there was a significant increment in the amplitude of FR during 60 minutes after drug administration (p<0.014). In conclusion, the serotonin receptor antagonists WAY100135 and Ritanserin increase the occurrence of FR activity as well as their duration and amplitude; in addition these results support the modulation of FR by serotonin and participation of the 5HT1a and 5HT2 receptors as possible mediators of its effect.

Disclosures: C.G. García-Barba: None. L. Medina-Ceja: None.

Poster

494. Epilepsy Mechanisms

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Topic: C.07. Epilepsy

Support: DoD USAMRMC Grant W81XWH-11-1-0502

Title: mTOR inhibition after controlled cortical impact alters hilar interneuron excitability

Authors: *C. R. BUTLER*¹, J. A. BOYCHUK¹,², B. N. SMITH¹,²,³;
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Abstract: Traumatic brain injury (TBI) is among the most common causes of acquired temporal lobe epilepsy (TLE). The latent period after injury and prior to expression of seizures includes plasticity events that support epileptogenesis, including cell loss and synaptic reorganization in the dentate gyrus. A murine model of TBI using controlled cortical impact (CCI) injury was used to examine the effect of daily rapamycin treatment (3 mg/kg) on excitability of surviving GABAergic hilar interneurons in mice that express GFP in a subset of inhibitory neurons (FVB-Tg(GadGFP)4570Swn/J; i.e., GIN mice). GFP-labeled hilar interneurons ipsilateral to CCI injury were reduced in number relative to controls, and rapamycin treatment did not inhibit this cell loss. Whole-cell patch-clamp and on-cell recordings in vitro were used to examine spontaneous

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EPSC frequency and action potential firing rates of surviving GFP-labeled hilar interneurons in GIN mice that were treated with rapamycin for 8-12 weeks after CCI injury. An increase in spontaneous EPSC frequency and action potential firing rate of GFP-labeled hilar interneurons ipsilateral to CCI injury was detected, relative to cells contralateral to the injury. Relative to CCI injury alone, daily rapamycin treatment resulted in a reduction in the increase in sEPSC frequency and spontaneous firing rate of GFP-labeled hilar interneurons ipsilateral to the injury. Although reduced relative to CCI injury, these measures were not normalized to control levels; analysis of the effects of high-dose rapamycin treatment (10 mg/kg) is underway. Rapamycin treatment therefore reduces the enhanced synaptic excitation of hilar interneurons after CCI injury in a manner consistent with suppression of reactive plasticity in granule cells. Ongoing experiments utilizing glutamate photolysis to activate granule cells and CA3 pyramids will test the hypothesis that effects of rapamycin treatment are mainly due to selective effects on mossy fiber sprouting.

Disclosures: C.R. Butler: None. J.A. Boychuk: None. B.N. Smith: None.

Poster

494. Epilepsy Mechanisms

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 494.26/G13

Topic: C.07. Epilepsy

Support: CIHR

Fondation Sainte Justine

Title: Exploring the role of p75NTR signaling pathway on GABAergic circuit maturation following neonatal hypoxia induced seizure

Authors: B. CHATTOPADHYAYA, M. BERRYER, D. DUFOUR-BERGERON, N. SANON, S. DESGENT, C. BOSOI, L. CARMANT, *G. DI CRISTO; Res. Ctr., CHU Ste.justine-University of Montreal, Montreal, QC, Canada

Abstract: Perinatal hypoxic-ischemic encephalopathy is the most important cause of acute mortality and morbidity in newborns. The most common acute effect of hypoxic-ischemic encephalopathy is neonatal seizures, which are very often refractory to conventional seizure medications. Hypoxia-induced seizures (HIS) are associated with a high incidence of epilepsy as well as cognitive disabilities later in life. Despite the significant long-term morbidity of HIS in
the neonates, there is currently no specific treatment. Further, our understanding of how HIS changes the developmental trajectory of neuronal circuit development, and ultimately results in epileptogenesis and cognitive dysfunction, is still mostly unknown. Understanding the precise mechanisms by which HIS affects brain development, and how its effects can be ameliorated, can help us in designing the appropriate therapeutic approaches towards preventing the consequences of neonatal hypoxia. Using a combination of molecular tools, electrophysiological recordings and imaging techniques, we studied the effects of HIS particularly on inhibitory GABAergic synapse development in rodent neocortex. In particular we determined that HIS affects the maturation and function of distinct GABAergic interneuron populations, parvalbumin (PV)-positive and somatostatin (SOM)-positive interneurons differentially in the neocortex and in the hippocampus. In particular, we show that PV expressing interneurons in the neocortex remain morphologically and functionally immature. This correlates with a reduction in gamma oscillation power during exploration, and with impaired working memory and social novelty recognition. We are currently investigating the molecular mechanisms involved, specifically looking at the role of neurotrophin receptor (p75NTR) mediated signaling pathways in ameliorating the deficits induced by HIS on GABAergic circuit development.


Poster

494. Epilepsy Mechanisms

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 494.27/G14

Topic: C.07. Epilepsy

Title: The over-expression of BDNF on adult neurogenesis and seizure vulnerability using a transgenic mouse model

Authors: *C. ISGOR, P. COOMBS, D. JOSEPH, K. GUTHRIE;
Charles E. Schmidt Biomed Ctrr, Florida Atlantic Univ., Boca Raton, FL

Abstract: Mice that overexpress brain-derived neurotrophic factor (BDNF) under the alpha-calcium/calmodulin-dependent protein kinase IIa promoter (termed TgBDNF mice) develop a mild cognitive deficit that is evident by 2-3 months of age that progresses to emergence of spontaneous seizures at ~6 months of age. Slow developing nature of behavioral disruptions observed in TgBDNF mice led to the hypothesis that chronic and sustained elevations in local BDNF are critical for progressive remodeling of hippocampal circuits implicated in
epileptogenesis. We have previously shown that the mossy fibres, axonal projections from the dentate gyrus granule neurons that innervate the CA3 field, are expanded in volume in TgBDNF mice compared to wildtype (WT) controls at 2-3 months of age prior to seizure development. The aim of this project is to determine whether alterations in normal adult neurogenesis in TgBDNF mice are antecedents to the onset of spontaneous seizures, and possibly contributing to the hyperexcitability of the circuitry. We compared the rate of adult neurogenesis in the TgBDNF mice to that observed in WT controls by use of bromodeoxyuridine labeling coupled with immunohistochemical staining for age-specific cell markers to identify proliferative pool size, neuronal differentiation and survival of new-born cells. Our preliminary analyses showed ~30% increase in the number of immature granule neurons in the TgBDNF compared to WT controls without a change in the number of proliferating cells, suggesting for increased number of adult-born granule neurons in BDNF enriched hippocampus. Furthermore we crossbred the TgBDNF mice with a strain that expresses the green fluorescent protein (GFP) under the GAD67 promoter transiently for the first 4 weeks after cell division. We will be assessing axonal and dendritic maturation rates of adult-born granule neurons in the TgBDNF mice using 3-D reconstruction of entire granule neurons. Understanding the mechanisms that contribute to the progression of seizures in this animal model will facilitate our ability to intervene and prevent adult-onset epilepsy of unknown causes. This work is supported by a collaborative FAU Seed Grant awarded to Drs. Isgor & Guthrie.


Poster

495. In vivo and In vitro Models of Acute and Chronic Seizures

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 495.01/G15

Topic: C.07. Epilepsy

Support: Ford Foundation Predoctoral Fellowship
        NSF GRFP
        Klingenstein Foundation
        Swebelius Family Trust

Title: Optically tracked interneuron dynamics during seizure initiation
Authors: M. L. MIRI¹, M. A. VINCK¹, J. A. CARDIN²;
¹Neurobio., ²Dept. of Neurobio., Yale Univ., New Haven, CT

Abstract: GABAergic inhibition is critical for regulation of excitation and maintains neural network stability through a precise balancing process. Loss or dysfunction of inhibitory interneurons is thought to lead to abnormal activity patterns and ultimately to seizure initiation. Two major GABAergic interneuron classes that may contribute to this process are the Parvalbumin-expressing (PV), fast-spiking cells and Somatostatin-expressing (SOM) cells. Using combined optical and electrophysiological tools, we investigate network dynamics during the development of seizures by monitoring the activity of individual interneurons and synchronization between multiple hippocampal cell types. Using AAV-DIO-ChR2-mCherry, we selectively targeted Channelrhodopsin-2 expression to PV or SOM interneurons in CA1. We used tetrode arrays to record the activity of many simultaneous cells in hippocampal CA1 in animals lightly anesthetized with ketamine/xylazine. We identified targeted ChR2-expressing PV and SOM interneurons in the recorded population by stimulating with brief pulses of blue light. Light pulses were continued intermittently throughout the experiment to track identified neurons without significantly altering the pattern of spontaneous activity. Using a pharmacological model of seizure induction (PTZ), we performed measurements of neural activity during baseline and multiple preictal periods. We assessed the pattern of spontaneous PV and SOM interneuron and excitatory neuron activity, changes in interneuron spike probability, and the temporal relationship between inhibitory and excitatory neuron activity during each period. We find that both PV and SOM cells exhibit a decrease in evoked spike probability during the transition from baseline to late preictal stages. To further characterize these progressive changes in spike probability, we measured the input-output function of ChR2-identified interneurons in response to a calibrated range of light intensities. We find a decrease in the slope of the input-output function as well as decrease in dynamic range for evoked spike probability for both PV and SOM IN populations leading up to seizure. Additionally, we find significant changes in the spike-triggered LFP average with respect to excitatory neuron firing during both early and late preictal periods. These results demonstrate the power of these combined approaches for dissecting network activity during seizure initiation and identify a complex pattern of alterations in interneuron activity that may contribute to the loss of E-I balance in the hippocampal network.


Poster

495. In vivo and In vitro Models of Acute and Chronic Seizures

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM
Program#/Poster#: 495.02/G16

Topic: C.07. Epilepsy

Support: EU FET Open 243914
   CFI LOF 28331
   CIHR OG 126137
   NSERC DG 418546-2
   CIHR New Investigator Award 288936

Title: An optogenetic kindling model of neocortical epilepsy

Authors: *E. CELA¹,², A. J. CHUNG¹, T. WANG¹, P. J. SJÖSTRÖM¹;
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Abstract: It is not well understood how otherwise healthy neuronal circuits become susceptible to seizures during epilepsy. To investigate local circuit restructuring associated with seizures, we developed a novel optogenetic animal model of epilepsy that is inspired by the classical kindling paradigm. We hypothesized that repeated and persistent simultaneous activation of a group of excitatory neurons should eventually elicit seizures in otherwise healthy mice. We expressed Channelrhodopsin-2 (ChR2) in primary motor cortex (M1) of male C57BL/6J mice by stereotactically injecting AAV-CaMKIIa-hChR2-E123T/T159C-p2A-EYFP bihemispherically. We allowed 21 days for recovery and ChR2 expression, after which animals were kindled by repeatedly illuminating M1 in 3-second-long 50-Hz burst every 48 hours using a 445-nm laser. Animals were monitored by EEG and video during each session. In 6 out of 6 animals, seizures progressively developed after 15.3±2.1 sessions. We defined seizures as EEG power exceeding background levels by two standard deviations for longer than 3 seconds. Seizure duration was quantified by EEG, and severity by a revised Racine scale. We found that seizure duration (r=0.52, p<0.001, n=4) and severity (r=0.59, p<0.001, n=4) increased with session, while seizure threshold was decreased (r=0.59, p<0.001, n=4). Additionally, the number of seizures also increased with session (r=0.48, p<0.001, n=6). We next examined if animals rekindled after a 36-day-long pause retained their high seizure susceptibility. Indeed, seizures had higher Racine score (p<0.05, n=5) and lasted longer (p<0.01, n=4). The seizure threshold was also lowered (p<0.01, n=4), and the number of sessions until first seizure was reduced (p<0.05, n=4). Finally, preliminary immunohistology for NeuN and GFAP indicated that there was no major neuronal loss or appreciable glial activation. In summary, we found that repeated optogenetic stimulation of awake behaving animals eventually elicited seizures in the absence of gross brain damage, and that animals retained an elevated seizure susceptibility for weeks. As our model allows for the
identification of directly activated cells, it enables the investigation of the role of specific cell populations in epileptogenesis.


Poster

495. *In vivo* and *In vitro* Models of Acute and Chronic Seizures

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 495.03/G17

Topic: C.07. Epilepsy

Title: Direct imaging of calcium pathology preceding kainic acid induced seizure activity in freely behaving mice

Authors: T. K. BERDYYEVA1, L. ALUISIO2, S. OTTE3, *R. M. WYATT1, C. DUGOVIC1, J. SHELTON1, K. GHOSH2, M. J. SCHNITZER2,3, T. LOVENBERG1, P. BONAVENTURE1; 1Janssen Res. and Develop., San Diego, CA; 2Inscopix, Palo Alto, CA; 3Stanford Univ., Palo Alto, CA

Abstract: The changes in calcium dynamics that precede convulsive states are unknown. Here we used calcium imaging combined with electroencephalographic measures (EEG) to study changes in calcium levels in the CA1 region of hippocampus following kainic acid administration (15 mg/kg) in freely behaving mice. Fluorescence changes in the GCaMP6f calcium sensor expressed under the CaMKII promoter were measured using a miniature fluorescence microscope directly attached to the head of the animal. We first demonstrated that the imaging procedure did not impact the seizure threshold. Calcium dynamics were studied specifically during non-convulsive states, where we observed that kainic acid treatment led to massive prolonged increases in calcium sensor fluorescence that were >10 fold greater in amplitude and >100 fold greater in duration than changes observed after vehicle administration. Such spreading (~1 mm/min) increases in fluorescence (“calcium waves”) were visible in the entire field of view (~1 mm2) and were preceded by a series of brief full field spikes in calcium fluorescence that escalated both in frequency (2-10 Hz) and intensity (1.5-10 fold brighter relative to baseline) and were geometrically and temporally distinct from the calcium waves. The first onset of pathological calcium events temporally coincided with the first, and usually brief, (<3 min) onset of a bout of epileptiform spikes (Stage 2, or spike-wave complexes, SWC), as assessed via simultaneous EEG recordings. Subsequent pathological calcium events re-occurred in >60% of the animals and were usually concordant with epileptiform EEG spikes. Similar calcium pathologies were observed in animals treated with different pro-convulsant agents.
(NMDA or PTZ), suggesting that the described calcium abnormalities were signatures of seizure activity rather than other potential pathological actions specific to kainic acid. Comparable calcium abnormalities were also observed in animals pre-treated with the anticonvulsant drug valproate (300 mg/kg), despite a reduction in the behavioral severity of the seizures. Prolonged exposure to abnormally high calcium concentrations that anticipate seizure activity in the absence of convulsive manifestations could be the basic mechanism underlying detrimental brain reorganizations and cognitive decline not only in epileptic patients, but also in other patients with similar metabolic insults. Therefore, direct visualization of calcium dynamics provides valuable insights into mechanisms of brain pathologies inaccessible by commonly used traditional methods.

**Disclosures:**
- **T.K. Berdyyeva:** Employment/Salary (full or part-time); Janssen Research & Development, LLC.
- **L. Aluisio:** Employment/Salary (full or part-time); Janssen Research & Development, LLC.
- **S. Otte:** Employment/Salary (full or part-time); Inscopix.
- **R.M. Wyatt:** Employment/Salary (full or part-time); Janssen Research & Development, LLC.
- **C. Dugovic:** Employment/Salary (full or part-time); Janssen Research & Development, LLC.
- **J. Shelton:** Employment/Salary (full or part-time); Janssen Research & Development, LLC.
- **K. Ghosh:** Employment/Salary (full or part-time); Inscopix.
- **M.J. Schnitzer:** Employment/Salary (full or part-time); Janssen Research & Development, LLC.
- **T. Lovenberg:** Employment/Salary (full or part-time); Janssen Research & Development, LLC.
- **P. Bonaventure:** Employment/Salary (full or part-time); Janssen Research & Development, LLC.

**Poster**

495. *In vivo* and *in vitro* Models of Acute and Chronic Seizures

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 495.04/G18

**Topic:** C.07. Epilepsy

**Title:** An integrated platform for behavioral and electrophysiological phenotyping: Application to an epilepsy mouse model

**Authors:** J. PANG1, *D. VOLFSON2-1, Y. PI1, J. DUERR1, S. M. O’NEILL1, T. SAMAD1, L. SCOTT1, D. L. BUHL1;
1Pfizer, Cambridge, MA; 2Pfizer, Canton, MA

**Abstract:** Understanding of electrophysiological correlates to behavior is important for the development of biomarker strategies and high-throughput assays for drug development. We report on the development of an integrated platform, which couples intelligent video monitoring
with electrophysiological measurements. We have applied a supervised machine learning approach to establish an automated video analysis system. To accomplish this, we employed a combination of static and dynamic features extracted from the video streams to segment episodes of distinct behavior such as walking and grooming, as well as disease-specific manifestations such as epileptic seizures. We then used synchronized electroencephalography (EEG) to derive event-locked signatures and establish context-dependent associations between behavior and electrophysiological endpoints. Here, we demonstrate the utility of the platform using the KA-induced epilepsy model in WT and GFAP-IL6 transgenic mice. To build an accurate behavioral assessment combined with EEG, multi-angle video streams coupled with EEG recordings from the cortex and CA1/CA3 regions of the hippocampus were synchronized with sub-second accuracy. We then developed a supervised learning approach that enabled robust identification of seizure subtypes defined according to Racine scale, along with the identification of EEG signatures for both convulsive and sub-convulsive seizures. In addition, we assessed morphology and statistical properties of ictal, preictal, and interictal spike events modulated by KA dosage and genotype. Our work demonstrates how a platform for automated behavioral and electrophysiological phenotyping could be used to study clinically translatable endpoints for models of chronic epilepsy, in which both sub-convulsive and convulsive activity must be continuously measured for extended periods of time.


Poster

495. In vivo and In vitro Models of Acute and Chronic Seizures

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 495.05/G19

Topic: C.07. Epilepsy

Support: CURE Grant 04-015-64 UP 2BT70

Title: Murine model of post-malarial epilepsy

Authors: *P. SSENTONGO1, A. ROBUCCIO1, D. G. SIM2, G. THUKU1, A. NABI1, F. G. GILLIAM3, S. L. WEISTEIN4, F. BAHARI1, B. SHANMUGASUNDARAM1, K. SHORT1, M. W. BILLARD1, E. C. PRICE1, P. J. DREW1, J. A. STOUTE3, A. F. READ2, B. J. GLUCKMAN1, S. J. SCHIFF3; 1CENTER FOR NEURAL ENGINEERING, 2INFECTIOUS DISEASES, PENNSYLVANIA
STATE UNIVERSITY, UNIVERSITY PARK, PA; ³MEDICINE, PENNSYLVANIA STATE UNIVERSITY, HERSHEY, PA; ⁴CHILDREN'S NATIONAL MEDICAL CENTER, DC, WA

Abstract: It is well established – though relatively unknown – that cerebral malaria (CM) leads to epilepsy. For the nearly 500,000 children who survive CM per year in sub-Saharan Africa, the estimated epilepsy rate after two-years is approximately 16%. Worldwide, this corresponds to the initiation of approximately 300,000 new cases of potentially preventable epilepsy per year. We investigated murine models of CM for evidence of post-malarial epilepsy and similarities to human CM. A post-malarial epilepsy model would serve both to identify critical mechanisms of damage, and provide a platform for investigation of adjunctive therapy. We investigated four combinations of different mixtures of mouse strain (C57BL/6, CBA, and Swiss-Webster SW) and Plasmodium-berghei (Pb) parasites (NK65 and ANKA). Eight cohorts of three-week old littermates were inoculated with parasitized erythrocytes. We rescued animals with Artesunate when they demonstrated signs of advanced CM. Age matched controls were inoculated with non-parasitized erythrocytes, and received identical drug treatment time matched to the infected animals. We developed a chronic recording system for long-term monitoring of brain and heart dynamics with DC sensitivity. Animals were implanted with EEG, EMG and ECG electrodes 14 days post treatment, and video-EEG continuously monitored for up to 8 months per animal. EEG data was analyzed for focal and generalized seizures. Of the mice surviving to recording: 88% (15/17) SW-PbNK65 had a uniform mixture of cortical, hippocampal, and primary generalized seizures, with median latency of 95 days to first convulsive seizure; 83% (10/12) SW-PbANKA had seizures with a predilection for focal hippocampal origin, with median latency of 36 days; 53% (8/15) C57BL/6-PbANKA developed epilepsy, with a high frequency of interictal generalized epileptic spikes, cortical secondarily generalizing seizures, and some hippocampal focal seizures, with a median latency of 40 days; 75% (3/4) CBA-PbANKA developed epilepsy with a predominance of primary generalizing seizures with median latency of 36 days. No seizures were observed from the control animals. We have developed multiple models of post-CM epilepsy that display various characteristics seen in human epilepsy. These models have high potential to enable us to, for the first time, explore physiological mechanisms that underlie epileptogenesis in humans that survive CM, and to develop rational adjunctive therapies based on these mechanisms to reduce this incidence.


Poster

495. In vivo and In vitro Models of Acute and Chronic Seizures

Location: Hall A
**Title:** Histological analysis for a murine model of post-malarial epilepsy

**Authors:** *A. ROBUCCIO*¹, P. SSENTONGO¹, D. SIM², A. GERONIMO¹, J. BACCON³, E. C. PRICE¹, F. BAHARI¹, A. F. READ², S. J. SCHIFF¹, B. J. GLUCKMAN³;
¹Ctr. for Neural Engin., ²Infectious disease, Pennsylvania State Univ., University Park, PA; ³Pathology and Lab. Medicine, Divisions of Anatom. Pathology and Exptl. Pathology, Pennsylvania State Univ. Col. of Med., Hershey, PA

**Abstract:** It is well established - though relatively unknown - that cerebral malaria (CM) leads to epilepsy. For the nearly 500,000 children who survive CM per year in sub-Saharan Africa, the estimated epilepsy rate after two-years is approximately 16%. Worldwide, this corresponds to initiation of approximately 300,000 new cases of potentially preventable epilepsy per year. A post-malarial epilepsy model would serve both to identify critical mechanisms of damage, and provide a platform for investigation of adjunctive therapy. We investigated all combinations of three mouse strains (C57BL/6, CBA, and Swiss-Webster SW) and two Plasmodium berghei (Pb) parasites (NK65 and ANKA) during the infection for their similarities to human CM. Cohorts of three-week old littermates were inoculated with parasitized erythrocytes from homologous donors. Age and sex-matched controls from each litter were inoculated with non-parasitized erythrocytes. Peripheral blood parasitemia was tracked daily. Animals were sacrificed on day 6 or 7 for histology when the animals displayed signs of severe CM. Human CM neuropathology hallmarks include adhesion and sequestration of infected red blood cells (iRBC) in brain microvessels, ring hemorrhages, microvascular thrombosis, and brain edema. Brains from all infected animals showed signs of severe edema, including tissue separation around blood vessels not seen in controls. Stereological methods were used to quantify densities of red blood cells (RBC), white blood cells (WBC), iRBC, and hemorrhages in subdivisions of the hippocampus and entorhinal cortex, especially to quantify different patterns of sequestration and focal ischemia in comparison with human CM. Our primary findings included that total blood cell densities were as much as 4 times higher in infected SW models compared to SW controls. Although hemorrhages were observed, they did not account for more than a few percent of this increase. The commonly used sequestration index (ratio of brain to peripheral parasitemia) did not accurately reflect the observed degree of sequestration. White blood cell densities were elevated 5-10 times over the controls throughout the brain for all models, reflective of a severe inflammatory response. We used these histological findings to guide our choice of models to investigate with chronic recordings for post-malarial epilepsy.

Poster

**495. In vivo and In vitro Models of Acute and Chronic Seizures**

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 495.07/G21

Topic: C.07. Epilepsy

Support: CURE Epilepsy. "A Murine Model for Preventing Postmalarial Epilepsy"

Title: Evidence of SUDEP in a murine model of Post-Malarial Epilepsy

Authors: *F. BAHARI*¹,², P. SSENTONGO³, D. G. SIM⁴, F. G. GILLIAM⁵, S. L. WEINSTEIN⁶, A. ROBUCCIO³, E. C. PRICE¹, A. NABI¹, B. SHANMUGASUNDARAM¹,², M. W. BILLARD¹,², P. J. DREW³,², A. READ⁴, S. J. SCHIFF³,², B. J. GLUCKMAN¹,²;

Abstract: It is well established - though relatively unknown - that cerebral malaria (CM) leads to epilepsy. For the nearly 500,000 children who survive CM per year in sub-Saharan Africa, the estimated epilepsy rate after two years is approximately 16%. Worldwide, this corresponds to initiation of approximately 300,000 new cases of potentially preventable epilepsy per year. We investigated murine models of CM for evidence of post-malarial epilepsy by combining four mouse strains and two Plasmodium berghei (Pb) parasites (NK65 and ANKA): Swiss-Webster SW-PbNK65, SW-PbANKA, C57BL/6-PbANKA, and CBA-PbANKA. Cohorts of three-week old littermates were inoculated with infected erythrocytes. We rescued animals with Artesunate when they demonstrated signs of advanced CM. Controls were inoculated with uninfected erythrocytes. We developed a chronic recording system for long-term monitoring of brain and heart dynamics with DC sensitivity. Animals were implanted with EEG, EMG, and ECG electrodes 14 or more days post treatment, and video-EEG monitored continuously for 1-8 months per animal. In all model combinations studied, recurrent spontaneous seizures were observed in a large fraction (50-90%) of the animals that survived to recording. Post treatment death rates prior to implant were observed in some mixtures, with the largest rate in SW-PbANKA. In these cases, death was accompanied by typically a single seizure-like event followed either by immediate death or a severe decrement in health. We recorded many seizure-
related deaths including 5/13 SW-PbANKA and 8/20 SW-PbNK65. All epileptic mice showed significant changes in cardiac activity associated with seizures. In 80% of the seizures, a transient preictal episode of bradycardia occurred, followed by ictal and late-ictal tachycardia. This was accompanied by a prevalence of missed beats (66%) before ictal activity. In contrast, no spontaneous seizures were observed in recordings of control animals, all of which demonstrated normal heart activity. Our findings suggest that repeated seizures cause or exacerbate serious cardiac pathologies and point to an autonomic nervous system disorder that later reinforces the epileptic state. These observations are consistent with what is currently known about the human condition of sudden unexplained death in epilepsy (SUDEP). These post-CM murine combinations therefore provide a platform for the study of the mechanisms of SUDEP and models to investigate interventions. Such interventions are critical to reduce the SUDEP risk in the estimated 1.8-2.4 million children that survive CM per year.


Poster

495. In vivo and In vitro Models of Acute and Chronic Seizures

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 495.08/G22

Topic: C.07. Epilepsy


Title: Activation of mTOR signaling pathway is secondary to neuronal excitability in a mouse model of mesial-temporal lobe epilepsy

Authors: *N. NITTA1,2, F. SUZUKI3, A. SHIMA3, K. NOZAKI3, A. DEPAULIS4; 1Neurosurg., Otsu/Shiga, Japan; 2Neurosurg., Shiga Univ. of Med. Sci., Otsu/Shiga, Japan; 3Neurosurg., Koto Mem. Hosp., Higashiomi, Japan; 4Grenoble-Institut des Neurosciences, Grenoble, France

Abstract: Objective Recent studies in animal models have suggested that the mammalian target of rapamycin (mTOR) signaling pathway is involved in several features of mesial-temporal lobe
epilepsy (MTLE). Here, we addressed whether mTOR activation promotes MTLE epileptogenesis or rather mediates neuroplasticity associated with hippocampal sclerosis. Method Male C57/bl6 mice were injected with KA (1 nmol) in the dorsal hippocampus and were treated with vehicle or rapamamycin (80mg/kg i.p. 5h after KA injection then 40 mg/kg/day i.p. from 1 to 20 days after KA). Immunohistolabelling of p-S6, ZnT3 and NeuN was performed at 21 days post KA and electroencephalography was recorded in animals implanted with hippocampal electrodes for 3 h at 25 days post KA. Results In mice injected intrahippocampally with kainate (1 nmol) we showed a biphasic increase of phospho-S6 ribosomal protein expression, the downstream product of mTOR signaling pathway, in dispersed granule cell layer of dentate gyrus (GCL) with a second phase lasting up to 6 months. Chronic treatment with rapamycin suppressed p-S6 expression, granule cell dispersion and mossy fiber sprouting but did not reduce hilar cell loss nor the development of hippocampal paroxysmal discharges. Neuronal inhibition by midazolam (2x10mg /Kg, i.p. at 21 days post KA) abolished the increased expression of p-S6 in dispersed GCL. Conclusion Our data suggest that activation of mTOR signaling pathway results from the increased neuronal excitation that develops in hippocampus and contribute to MTLE morphological changes in this structure. However, they do not support the role of this pathway in the development of MTLE nor its use as a therapy for this form of epilepsy.


Poster

495. *In vivo* and *In vitro* Models of Acute and Chronic Seizures

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 495.09/G23

Topic: C.07. Epilepsy

Support: NIH Grant NS058674

NIH Grant NS070824

Title: Brain site-specific suppression of glutamine synthetase in mice using an adeno-associated virus knockout approach

Authors: *H. WANG¹, R. DHAHER¹, M. FARINA¹, Y. ZHOU², S.-P. YEE³, N. C. DANBOLT², T. EID¹;

Abstract: The expression of astroglial glutamine synthetase (GS), a key enzyme in the glutamate/GABA-glutamine cycle, is perturbed in brain disorders such as Alzheimer's disease, schizophrenia, depression, and mesial temporal lobe epilepsy (MTLE). In human MTLE, GS is lost from astrocytes in specific subfields of the hippocampal formation; however, the consequences of such a loss are not fully understood. Here, we used either hippocampal infusion of the GS inhibitor methionine sulfoximine (MSO) or adeno-associated virus (AAV) to assess the consequences of GS suppression in C57Bl/6J mice. Conditional knockouts for GS were generated in C57Bl/6J mice by insertion of loxP sites at the front of the 2nd and 7th exon in the GS gene. Delivery of Cre recombinase to floxed-GS mice (10-12 weeks) was mediated by AAV5-Cre-GFP delivered unilaterally into the hippocampus. In the contralateral hippocampus, control AAV5-GFP virus was delivered. On both sides, a total of 0.5 μL of virus with a titer of ~1x10^{12} was delivered. As controls, wild-type mice (10-12 weeks) were injected following the same protocol. Separate groups of wild type mice (10-12 weeks) were implanted with an osmotic pump that infused either MSO or phosphate buffered saline (PBS) unilaterally into the hippocampus. Video-intracranial electroencephalogram (EEG) recording was performed immediately following placement of the pump, and 4 weeks after injection of virus, to monitor for seizures. Severity of seizures was characterized using a modified Racine Scale. Mice were perfusion fixed with 4% paraformaldehyde after 2-3 weeks of EEG monitoring. Brains were horizontally sectioned at 40 μm with a Vibratome. Virus injected sections were co-labeled for GS and GFP to assess GS levels. All of the MSO-infused mice and none of the PBS-infused mice developed spontaneous recurrent seizures including convulsive, behavioral seizures. In the ipsilateral hippocampus, mice exhibited glial proliferation and patterned loss of neurons, whereas minimal brain injury was present elsewhere. In the floxed-GS mice, GS levels were decreased in the Cre-injected side at 4-5 weeks after viral delivery whereas minimal reduction in GS was noted in the control injected side. These findings suggest a key role of GS suppression in the causation of MTLE and provide useful laboratory tools for further investigation on the role of GS in health and disease.


Poster

495. In vivo and In vitro Models of Acute and Chronic Seizures

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM
**Program#/Poster#:** 495.10/G24

**Topic:** C.07. Epilepsy

**Support:** NIH GRANT PA-13-302

**Title:** Seizure progression in a PTEN KO model

**Authors:** *S. KHADEMI*
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**Abstract:** Temporal lobe epilepsy is one of the more common and difficult to treat of adult epilepsies. Patients with temporal lobe epilepsy can have simple partial seizures, in which consciousness is preserved, or complex partial seizures in which consciousness is lost during the event. Patients can exhibit one or both types of seizures, but importantly, some patients will transition from simple to complex seizures, indicative of increasing disease severity. The mechanisms regulating disease progression are unknown. To explore potential mechanisms of epilepsy progression, we have developed a novel genetic mouse model of epilepsy in which deletion of phosphatase and tensin homologue (PTEN) from a subset of late-generated hippocampal dentate granule cells produces an epilepsy syndrome. The percentage of granule cells in which PTEN is deleted from can be experimentally controlled in this model. We have now demonstrated that PTEN deletion from >20% of granule cells in three-week-old mice leads to the development of spontaneous complex seizures about nine weeks later; evident as seizure spread to motor cortex. For the present study, we will test the hypothesis that the seizure phenotype in these animals is regulated by the percentage of PTEN KO cells. We predict that PTEN deletion from fewer cells will produce simple partial, rather than complex partial seizures - the latter evident as focal hippocampal seizures. Accumulation of abnormal granule cells occurs in a number of epilepsy models, and if seizure phenotype is dependent on abnormal granule cell number, this accumulation could account for disease progression in some epilepsy patients. If correct, preventing this accumulation might prevent disease worsening.

**Disclosures:** S. Khademi: None.

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**Poster**

495. *In vivo* and *in vitro* Models of Acute and Chronic Seizures

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 495.11/G25

**Topic:** C.07. Epilepsy
**Support:** Alberta Children’s Hospital Research Institute
Alberta Children’s Hospital Foundation

**Title:** Spontaneous recurrent seizures and hippocampal structural pathology in Ndel1 conditional knockout mice

**Authors:** *C. N. GAVRILOVICI*1, Y. JIANG2, M. CHANSARD2, F. GAO3, R. H. LIU2, K. PARSONS2, S. K. PARK2, R. TOBIAS1, L. SCOTT1, I. KIROSKI2, G. C. TESKEY3, L. H. TSAI5, J. M. RHO7, M. D. NGUYEN4;


**Abstract:** Surgically-resected tissues from patients with epilepsy at times exhibit disruption of the neuronal cytoskeleton, and mutations in genes encoding components of the cytoskeleton - critically involved in neuronal network development - can lead to malformations of cortical development and medically intractable epilepsy. However, it is unknown whether postnatal modification of cytoskeletal structure and/or function can lead to epilepsy. Here, we generated a conditional knockout mouse of the microtubule-associated/signaling protein Ndel1 (Ndel1 CKO, by breeding CaMKIIα-Cre transgenic mice with Ndel1-LoxP mice) in forebrain excitatory neurons, and tested the hypothesis that postnatal dysregulation of the cytoskeleton results in aberrant hippocampal structure and neuronal excitability. Confocal/electron microscopy, video-EEG, molecular, cellular and behavioral techniques were used to study CA1 hippocampus in Ndel1 CKO and WT mice. Genome-wide transcriptome profiling of hippocampi derived from both CKO and control mice was performed using RNA sequencing. Ndel1 CKO mice exhibit spontaneous recurrent seizures (SRS), frequent interictal spikes and die prematurely (~10.5 weeks). Further, CKO mice display hippocampal lamination defects (i.e., misalignment of CA1 pyramidal [pyr] cells). This CA1 dysplasia also involves primitive pyr cell dendritic arbors, atrophied spines, ~48% reduction in the number of asymmetric synapses and ~55% reduction in the number of pyr cell dendritic microtubules (P<0.01). These structural abnormalities are paralleled by increased excitability of CA1 pyr cells: ~26% increase in input resistance, ~35% decrease in threshold current and ~51% enhanced firing (P<0.05). Further, CKO have interneuron defects, including: ~54% reduction in the number of CA1 symmetric synapses and ~40% decrease in the frequency of mIPSCs recorded in pyr cells (P<0.01). Finally, genome-wide transcriptome analysis of CKO hippocampi revealed deregulation of genes associated with human epilepsy that can be normalized (rescued) with Reelin treatment. In summary, we have identified several postnatal changes in hippocampi of Ndel1 CKO mice that likely contribute to...
SRS, specifically CA1 dendritic/synaptic pathologies, postnatal CA1 pyr cell dispersion and hyperexcitability, and an interneuronopathy. The identification of genes linked to epilepsy combined with morpho-functional abnormalities in Ndel1 CKO mice suggest that Ndel1 may represent an important target for the study of epilepsy and advances the notion that the postnatal disruption of the cytoskeleton may be an important determinant of the epileptic state.


Poster

495. *In vivo* and *In vitro* Models of Acute and Chronic Seizures

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 495.12/G26

Topic: C.07. Epilepsy


Title: Modification of the focal kainate model in rats for the use in pharmacological studies on antiepileptogenesis

Authors: R. KLEE1,2, C. BRANDT1,2, K. TÖLLNER1,2, *W. LOSCHER1,2; 1Univ. of Vet. Med. Hannover, Hannover, Germany; 2Ctr. for Systems Neurosci., Hannover, Germany

Abstract: A large variety of brain insults, including a status epilepticus (SE), can induce the development of symptomatic epilepsies, particularly temporal lobe epilepsy (TLE). In the latent period after the initial insult multiple molecular, structural, and functional changes, called epileptogenesis, proceed in the brain. All these mechanisms can lead to spontaneous recurrent seizures (SRS). An urgent medical need is to develop antiepileptogenic strategies that modify or even prevent the development of SRS. In rodent post-SE models, kainate, a neurotoxic glutamate analogue, is widely used to induce SE for the investigation of epileptogenesis and TLE. Focal injection of kainate in the hippocampus induces an epileptic focus and alterations in the brain that are similar to human TLE. In mice, the chronic epileptic state in the focal kainate model is characterized by a high frequency of seizure-like events (SLEs) in EEG of the ipsilateral hippocampus and infrequent generalized convulsive seizures. In rats, only the infrequent generalized convulsive seizures are observed. The rat model is therefore much more time-
consuming and less suitable for investigating the antiepileptogenic potential of different drugs and their combinations. An advantage of the focal kainate model in rats is a high face validity with respect to human TLE. The reason for this species difference is not known, but the dose of kainate and the localization of kainate injection in the hippocampus may be involved. For this reason we modified the focal kainate model in rats and adapted it accordingly to the focal kainate model in mice. We injected kainate under anaesthesia, implanted an electrode in the hippocampal focus and changed step by step the injection site, the dose, and the volume of the kainate injection. Preliminary results indicate that neither the change of injection site in the hippocampus nor the increase of the kainate dose or volume lead to similar SLE frequencies as seen in mice. These findings support the conclusion, that it is not possible to mimic the advantages of the mouse model in rats, and therefore it remains more time-consuming to study antiepileptogenic effects in the rat model.


Poster

495. *In vivo* and *In vitro* Models of Acute and Chronic Seizures

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 495.13/G27

Topic: C.07. Epilepsy

Support: Academy of Finland

Title: mir-124 as a potential biomarker of traumatic brain injury

Authors: *N. VUOKILA*¹, N. PUHAKKA¹, K. LUKASIUK², A. PITKANEN¹;
¹A. I. Virtanen Inst. for Mol. Sci., Univ. of Eastern Finland, Kuopio, Finland; ²Nencki Inst. of Exptl. Biol., Warsaw, Poland

Abstract: Traumatic brain injury (TBI) is one of the most common reasons for acquired epilepsy. Not all TBI patients develop seizures. We hypothesize that certain miRNAs are involved in post-traumatic epileptogenesis and investigation of changes in the expression level of miRNAs would provide both biomarkers and possible drug targets. TBI was induced to adult male Sprague-Dawley rats using lateral fluid-percussion. The miRNA of interest was determined with Ingenuity Pathway Analysis (IPA) using Affymetrics array data (p<0.05) derived from dentate gyrus 3 months post-TBI. To investigate networks related to the chosen miRNA we performed STRING analysis. Other targets of miR-124 were determined with database search using miRWalk, miRanda, miRDB, RNA22, TargetScan, DIANmiT, PICTAR4, PICTAR5 and
PITA. Results from STRING and miRWalk were compared to transcriptome profiling with Gene Enrichment Analysis (GSEA). Circulating miR-124 was detected from whole blood samples at 2 days and 2 months post-TBI using RT-qPCR to investigate it as a possible biomarker. The results from blood RT-qPCR were analysed with receiver operating characteristic (ROC) test. IPA predicted miR-124 to be down-regulated as 18 of its targets were upregulated (z-score=-4.176, p<0.01). STRING analysis connected these targets to Jak/STAT pathway, APP processing and ubiquitination. In GSEA 31% (32/104, FDR<0.01) of the additional molecules suggested by STRING and 18% (57/321, FDR<0.05) of targets predicted by database search were enriched in transcriptome data. According to RT-qPCR the amount of circulating miR-124 at 2 day post-TBI seems to be elevated (189% compared to control, p=0.0500). ROC analysis indicates that at that timepoint miR-124 is a potential biomarker (area=0.803, p<0.05). At 2 months post-TBI no change was found when compared to control. Gene expression data suggests that miR-124 regulates pathways previously related to epilepsy. ROC analysis indicated that the elevated blood miR-124 content at 2 days post-TBI could be used as molecular biomarker for TBI.

Disclosures: N. Vuokila: None. N. Puhakka: None. K. Lukasiuk: None. A. Pitkanen: None.
clinically available ‘antiepileptogenic’ drugs to prevent the development of epilepsy after an epileptogenic brain injury. Thus, the development of novel drugs to prevent or modify epilepsy in patients at risk is an urgent medical need. The intrahippocampal kainate model in mice, a post-SE model of mesial temporal lobe epilepsy, seems to be an ideal screening model in the search for antiepileptogenic drugs. Several groups reported that in male mice (Swiss or C57BL/6) a kainate induced SE is followed by a short latent period (days to weeks) after which focal seizures occur at a very high frequency, so that an antiepileptogenic drug effect can easily be detected without the need of long continuous video/EEG recording. In our hands - using female NMRI mice - no latent period could be observed. Therefore, we tried to modify the model in female mice by decreasing the kainate dose (from 1.0 nM stepwise down to 0.25), changing the location of the EEG electrode (hippocampus vs. cortex), changing the anesthetic used during kainate injection (chloral hydrate vs. isoflurane), or increasing the age at time of kainate injection (mid-adolescent to post-adolescent), but none of these modifications yielded a latent period - until we switched to male mice. A clear latent period could only be seen in male NMRI mice but not in female mice of three different mouse strains (NMRI, C57BL/6, FVB/N). Moreover, focal hippocampal paroxysmal discharges (HPDs), which are considered the typical focal seizure in this model, only occurred frequently in male mice but almost never in females of the examined mouse strains. The present data demonstrate for the first time marked sex-related differences in the latent period, which most certainly will affect the preclinical antiepileptogenesis study design and analyses of outcome measures.

Disclosures: K. Töllner: None. F. Twele: None. C. Brandt: None. W. Löscher: None.

Poster

495. *In vivo* and *in vitro* Models of Acute and Chronic Seizures

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 495.15/G29

Topic: C.07. Epilepsy


Title: Hippocampal volume and T2 signal changes in the pilocarpine model of temporal lobe epilepsy

Authors: *R. BARBOSA¹, A. S. VIEIRA², A. H. B. DE MATOS², B. M. DE CAMPOS¹, R. F. CASSEB¹, R. GILIOLI³, I. LOPES-CENDES², F. CENDES³;
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**Abstract:** Background: The limbic structures of the temporal lobe are more susceptible to brain injuries related to epileptogenesis, such as the ones observed in patients with temporal lobe epilepsy and animal models. However, some of these structural brain changes during the development of epilepsy are still unknown. Objectives: To analyze neuroimaging changes (volumetry and T2 signal) in the hippocampus within the first 30 days after pilocarpine induced status epilepticus (SE). Methods: A total of 10 adult male Wistar rats were submitted to magnetic resonance imaging (MRI) with acquisition of T2-weighted images using a volumetric coil with 8 integrated channels and resources for positioning and anesthesia (Rapid Biomedical GmbH, Wurzburg, Germany) in a 3 Tesla Philips scanner. Images were acquired prior to any intervention and then 48 hours, 15 days and 30 days after pilocarpine SE induction. These rats were pre-treated with methyl scopolamine (1mg/kg) to reduce systemic cholinergic side effects. Thirty minutes after, rats received a systemic injection of pilocarpine hydrochloride (380mg/kg) and after 4 hours of the onset of SE, (4 mg/kg) diazepam to interrupt crises. The analysis of behavioral seizures followed the classical parameters by direct visual inspection, according to the Racine scale. The anatomical boundaries of the hippocampus were defined by the brain atlas rats (Paxinos & Watson, 1986) and volumetric analysis was performed using the Display program. SE was observed between 1-2 hours after each injection. The SE-related mortality was 50% in this study. Results: Four out of 5 rats developed SE. Overall, the mean hippocampal volumes increased progressively after SE induction; however, there was a great individual variation. Considering rats which developed SE, one showed a small reduction of hippocampal volumes after 48 hours and all four rats had an increase of hippocampal volumes 15 days after inducing SE. Only 2 animals showed a further increase of hippocampal volumes in the MRI 30 days after SE. The animal that did not develop SE had a small reduction of hippocampal volumes 48 hours and 15 days after SE, and an increase of hippocampal volumes 30 days after SE. The visual assessment of T2-weighted images showed a progressive increase of signal intensity in hippocampus after inducing SE in all four rats with SE. The rat without SE after pilocarpine did not have T2 signal changes in the scans. Conclusion: The main finding of this preliminary longitudinal study confirms changes in hippocampus volumetry and T2 signal after SE induction by pilocarpine. Early changes in T2 hippocampal signal precedes volume changes and is related to the occurrence of SE.


**Poster**

**495. In vivo and In vitro Models of Acute and Chronic Seizures**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM
Program#/Poster#: 495.16/G30

Topic: C.07. Epilepsy

Support: NSF CAREER Award 1149446

Title: Respiration-induced seizures in the adult naked mole-rat

Authors: *M. ZIONS, T. DZEDZITS, D. MCCLOSKEY;
CUNY CSI, Staten Island, NY

Abstract: The African naked mole-rat (H. glaber) presents a unique opportunity to study the contributions of physiology and environment on neuronal synchrony and seizures. Besides their extreme longevity (> 30 years), and inducible reproductive and stress hormones (as cooperative breeders), naked mole-rats have a wide tolerance for ambient oxygen and carbon dioxide (as fossorial rodents), and a brain temperature that is tightly coupled to the environment (as poikilotherms). In addition, naked mole-rats appear to be prone to seizure, displaying sporadic spontaneous seizure behaviors in vivo and spontaneous hippocampal epileptiform burst discharges in vitro. However, the mechanisms underlying this lowered seizure threshold are not well understood. Here, we measured behavioral responses in response to hyperthermia (~41 C, ambient and rectal) and observed robust seizures into adulthood (>1 year). To determine whether these seizures were in response to brain temperature or alkalosis due to increased respiration (as reported by Kaila and colleagues in juvenile rat and mouse models of febrile seizures) we injected adult naked mole-rats with sodium bicarbonate (5 mM/kg i.p.) and seizures were confirmed on EEG and video. Administration of 5% CO2 in room air suppressed behavioral seizure and ictal spiking whereas restoration of normal CO2 was accompanied by renewed seizing. Nikethamide, a respiratory stimulant, was injected (100 mg/kg i.p.) and produced rapid, robust seizure activity in room air and temperature. Together, these results suggest that an enhanced sensitivity to brain alkalosis mediates the seizure susceptibility of this species, and posits the naked mole-rat as a new animal model in the study of febrile seizures.

Disclosures: M. Zions: None. T. Dzedzits: None. D. McCloskey: None.

Poster

495. In vivo and In vitro Models of Acute and Chronic Seizures

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 495.17/G31

Topic: C.07. Epilepsy
Support: Fapesp Process number 2011/10898-9
CNPq

Title: Ictal patterns in limbic and cortical circuitry in an epilepsy model induced by perforant pathway long-term stimulation

Authors: *A. S. BROGGINI¹, I. M. ESTEVES², R. N. LEÃO³, R. N. ROMCY-PEREIRA³, J. P. LEITE²;
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Abstract: Abnormalities in encephalography (EEG) (e.g. increase in focal slow activity and interictal spikes) are often found in focal epilepsy. Hence, EEG can be potentially useful for seizure prediction. Recently, studies have focused on measures of brain activity, such as the total spectral power and the synchronization between regions. At the molecular level, it is known that immediate expression genes (Zif268) are neurobiological tools widely used for functional activity mapping. Here, we characterize the spontaneous recurrent seizures (SRS) and perform spectral analysis of local field potentials (LFP) before and after seizures detected in a temporal lobe epilepsy (TLE) model with ictal activity restricted to the hippocampus (hippo). The TLE model is generated by long-term intermittent electrical stimulation of the perforant pathway (PP). LFPs were recorded in dentate gyrus (DG) of hippo and medial prefrontal cortex (mPFC) and SRS were monitored by video-EEG. The power spectrum and coherence in oscillations between hippo and mPFC were measured in 2 minutes segments before and after seizures for the following frequency bands: delta, theta, beta, low gamma and high gamma oscillations. Behavioral patterns in these segments were categorized in awake, sleeping or exploration states. Besides, we assessed Zif268 and parvalbumin expression 2 hours after SRS to map the activation/synaptic plasticity and death of inhibitory interneurons. Eight out of 16 stimulated animals displayed at least one electrographic seizure, predominantly in the afternoon. The mean latency of the first SRS was 37 days. In 33 electrographic SRS observed in a total of 14 animals, we found a significant decrease in theta power after SRS in comparison to segments before seizures. Mean coherence in theta (hippo-PFC) increased in the last 30 seconds preceding seizures. The largest increase in hipo-pfc coherence was observed in animals that were sleeping before SRS. Rats in exploration state did not show significant difference in coherence preceding seizures. Stimulated animals showed clear expression of Zif268 in mPFC, entorhinal cortex and DG regions, 2 hours after SRS. In CA1, CA3 and hilus, where the neuronal loss was intense, Zif268 staining was absent. In CA2, where neuronal loss was mild, weak Zif268 expression was observed. Parvalbumin expression also decreased in mPFC (pre-limbic area) and DG regions 2 hours following seizures, compared with control animals. Taken together, these results show that an increase in hipo/mPFC coherence may serve as a predictor of SRS. Further analysis will reveal whether these EEG predictors are also correlated to the extent of network wiring or cell damage.
Electrographic pattern of the kainic acid induced status epilepticus

Authors: F. M. NOÈ, C. ALESSI, A. CATTALINI, M. DE CURTIS, *V. GNATKOVSKY; IRCCS Inst. Neurologico C. Besta, Milan, Italy

Abstract: Status epilepticus (SE) is a neurological emergency associated with significant morbidity and mortality, characterized either by prolonged seizures or by a series of seizures, lasting over 30 min. In many epilepsy animal models, SE is assumed to trigger the epileptogenic process. While chronic seizure events in animal models had been well described, very few studies are available about electroencephalographic SE development and on the resulting epileptogenic process. In our study we focused on the acute phase (first 3 days) following Kainic Acid (KA)-induced SE in guinea pig model in vivo (Carriero et al., Epilepsia 2012; Arcieri et al., Epilepsia 2014). Seven days after electrode implantation bilateral in hippocampi, and frontal and parietal cortices, KA (5mM, 1 µl) was injected unilaterally in the dorsal CA1 area. Continuous video-EEG recording was performed starting 24 h before till 3 days after SE induction. A preliminary group of 11 animals was analyzed and two principal EEG patterns were observed. In 5 animals, acute seizures were recorded, followed by a continuous, high-amplitude rhythmic spike activity at 15-25 Hz lasting 8-20 h. In 4 animals, high amplitude and frequency discharges (HAFDs, up to 5 h) followed by a low-amplitude spike activity with lower frequency rate, lasting about 8-10 h, were recorded. Finally, in 2 animals atypical patterns were observed. A variable number of HAFDs (5-90) was observed exclusively during the first 6 h of SE. HAFDs were always characterized by post-ictal depression and by spiking activity recovering between consecutive episodes. Long-term EEG frequency analysis showed increased 11-30 Hz activity during SE. This activity slowly increased during the transition from the KA-induced acute ictal phase to the spike activity phase. Behavioral observation revealed that the seizures correlated
with immobility, stereotyped movements and tonic-clonic pattern, whereas the spike activity was characterized by a slow return to normal behavior. When diazepam (DZP; 12.5 mg/kg, i.p. for 90 min; n=4), was administered in order to prevent SE induction, acute seizures were blocked and a continuous spike activity at 15-25 Hz was observed. After DZP withdrawal, EEG SE was resumed in all animals, despite the obvious lack of motor clinical manifestations. Preliminary histological evidence suggests that also DZP-treated animals showed KA-induced damage associated to spiking activity. Conclusions: Different EEG patterns can be generated during KA-induced SE. KA injection mainly correlates with fast spiking activity at 15-25 Hz at the injection site. Seizures, but not fast activity can be blocked by DZP.

Disclosures: F.M. Noè: None. C. Alessi: None. A. Cattalini: None. M. de Curtis: None. V. Gnatkovsky: None.

Poster

495. In vivo and In vitro Models of Acute and Chronic Seizures

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 495.19/G33

Topic: C.07. Epilepsy

Title: Histology of Epileptogenesis in Zebrafish

Authors: *D. Q. PHAN1, M. BERBEROGLU2, C. BEATTIE2, C. W. HALL2;
1Dept. of Neurosci., Johns Hopkins Univ., Baltimore, MD; 2Ohio State Univ., Columbus, OH

Abstract: Characterized by recurrent unprovoked seizures, epilepsy is a chronic lifelong disorder affecting 1% of the general population associated with higher rates of mortality, cognitive impairment, and psychosocial dysfunction at an annual economic cost of $12 billion USD. Although epilepsy is mostly treated with medications, medical therapy is ineffective for 25% of patients and produces severe side effects, emphasizing the need to explore alternative therapeutic approaches. To that end, a better understanding of epileptogenesis is needed. Epileptogenesis is a complex and dynamic process by which brain tissues become prone to spontaneously initiating and sustaining electrical seizure activity. Epileptogenesis results from changes in the brain’s biochemical and micro-anatomic structures, including local inflammation, neurogenesis, and reactive gliosis. Each of these alterations is a potential therapeutic target for halting epileptogenesis, but little is known about which process or combination of processes would be optimum. Work in this area is slowed by the myriad of complicated and overlapping biological pathways involved, compounded by the relatively low throughput capacity of mammalian epilepsy models. The overarching goal of this study is to determine whether the
zebrafish epilepsy model can be used to study epileptogenesis. Like rodents, zebrafish have a completely tractable genome, vertebrate neural architecture, and are used to study many human diseases including epilepsy. The advantages of the zebrafish model over rodent models, especially as it pertains to epilepsy, include more cost effective generation and maintenance of transgenic lines and greater amenability to high throughput, stringently controlled experimentation. In our study, we found that drug-induced seizures caused epileptogenic histological transformations in the zebrafish brain, including inflammatory changes, increased neural proliferation and neurogenesis, and reactive gliosis. These findings lend support that the high throughput, cost-effective zebrafish epilepsy model may have utility in studying epileptogenesis.

Disclosures: D.Q. Phan: None. M. Berberoglu: None. C. Beattie: None. C.W. Hall: None.

Poster

495. *In vivo* and *In vitro* Models of Acute and Chronic Seizures

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 495.20/G34

Topic: C.07. Epilepsy

Title: Characteristics of a non-human primate model for electrically induced epileptic seizures

Authors: *J. MYLIUS*1, F. MARQUARDT1, A. Y. KITAY3, E. SELEZNEVA1, L. BUENTJEN4, C. KLUGE1, J. VOGES4, H.-J. HEINZE2, F. C. SCHMITT3, M. BROSCH1,5; 1Special Lab. Primate Neurobio., 2Dept. Behavioral Neurol., Leibniz Inst. for Neurobio., Magdeburg, Germany; 3Dept. of Neurol., 4Dept. of Stereotactic Neurosurg., Otto-von-Guericke Univ., Magdeburg, Germany; 5Ctr. for Behavioral Brain Sci., Otto-von-Guericke-University, Magdeburg, Germany

Abstract: For a better understanding of the pathophysiology of epilepsy in humans and the development of novel therapies, especially for patients with treatment resistant epilepsy, appropriate animal models are indispensable. Several animal models of epilepsy or seizure induction exist, e.g., chemically induced rodent models. However, they exhibit not negligible differences from the conditio humana, which hampers the transferability of results to humans. Therefore, we used long-tailed macaques as an animal model for experimental seizures, in which seizures are induced acutely by electrical stimulation of the motor cortex. Both stimulation and recording microelectrodes were placed intracortically into the ventral primary motor cortex. In addition to the intracortically recorded local field potentials, we obtained electrocorticograms from a surface electrode array above ventral primary motor cortex. To assess the influence of
stimulus parameters on the characteristics of the electrically induced epileptic activity, we varied the duration of the pulse trains from 1 to 4 seconds and the intensity from 10 to 500 µA. Pulse trains were applied with a constant frequency of 60 Hz. The epileptic activity was determined by the total number of epileptic spikes, their amplitude, and their frequency composition. Additionally, the animals were video monitored for clinically driven classification. This categorial seizure classification comprises 6 groups with increasing severity (from A: tonic and/or clonic convulsions of one muscle group to F: generalized tonic-clonic seizures). Three hundred forty stimulations were performed within 7 experimental sessions on different days in two monkeys. In about one fourth of these stimulations we were able to electrically induce epileptic activity, which was accompanied by clinical seizure manifestation in more than half of the cases. The number of epileptic spikes induced by one stimulus train and the duration of epileptic activity correlated with the intensity and the duration of stimulation. However, most strongly it depended on the applied charge, i.e., the product of current intensity and duration. With higher charge, longer epileptic seizures with higher clinical severity were induced. Here, we present a stable non-human primate model that allows repetitive and reversible induction of focal motor seizures. This model represents an excellent tool for preclinical testing of anti-ictal therapy modalities, such as deep brain stimulation, whose anti-ictal efficacy is based on the alteration of seizure propagation.


Poster

495. In vivo and In vitro Models of Acute and Chronic Seizures

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 495.21/G35

Topic: C.07. Epilepsy

Title: Evaluation of acetylcholine and neuropathology following the administration of nerve agent and potential neuroprotective drugs in freely moving rats


Abstract: Organophosphorus nerve agents such as soman (GD) inhibit acetylcholinesterase, producing an excess of acetylcholine (ACh) that results in hypersecretions and respiratory distress in the periphery. These nerve agents also produce status epilepticus that contributes to
Several drugs (topiramate, clobazam, pregnanolone, allopregnanolone, UBP 302, cyclopentyladenosine [CPA], ketamine, midazolam, and scopolamine) have been identified as potential neuroprotectants that may terminate seizures and reduce brain damage. To begin to understand if these drugs mitigate neuropathology following nerve agent exposure, this study employed striatal in vivo brain microdialysis and high performance liquid chromatography to respectively collect and analyze extracellular ACh in freely moving rats following GD exposure and drug treatment at 20 minutes after seizure onset. Along with the evaluation of ACh levels, EEG seizure activity was recorded and neuropathology assessed 24 hrs later. The average time for seizure onset was 5.15 minutes after GD administration. The average basal ACh level was 0.97 pmol/10µL. GD-exposed groups showed a marked increase of ACh, peaking at 30 minutes post-exposure to approximately 800% of control levels. ACh concentration then steadily decreased toward baseline levels. Approximately 30 min after treatment, only midazolam (10 mg/kg) and CPA (60 mg/kg) caused a significant reduction of ACh levels. This reduction persisted for 1 hour for midazolam-treated groups and until the end of the collection period for CPA. While CPA reduced ACh levels more rapidly than midazolam, both drugs facilitated a return to baseline levels at least 2.5 hrs after treatment. At 24 hrs, only animals treated with CPA (64%), midazolam (18%), and scopolamine (27%) exhibited seizure termination. All treatments with the exception of topiramate demonstrated a decrease in total neuropathology 24 hours after exposure. While clobazam, pregnanolone, allopregnanolone, UBP 302, and ketamine only increased neuroprotection by at most 20%, CPA, midazolam, and scopolamine increased neuroprotection by at least 59%. Our results suggest that delayed treatment with CPA, midazolam, or scopolamine is effective at reducing GD-induced seizure activity and neuropathology, with CPA and midazolam able to facilitate a reduction in GD-induced ACh elevation as well. This research was supported by an Interagency Agreement (IAA) between NIH/NIAID (IAA#AOD12058-001-00000) and the USAMRICD (#A120-B.P2012-02).

Disclosures: C. Acon-Chen: None. J. Koenig: None. G. Smith: None. A. Truitt: None. T. Thomas: None. T. Shih: B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; NIH.

Poster

**495. In vivo and In vitro Models of Acute and Chronic Seizures**

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 495.22/G36

Topic: C.07. Epilepsy
Support: DoD Grant PR121769

Title: Somatostatin reverses kindling-induced increases in type-1 progenitor cells in the dentate gyrus of adult rats

Authors: *J. A. LEIBOWITZ1,2, G. NATARAJAN1,3,4,5, M. A. KING1,6,7, P. R. CARNEY1,3,4,5, B. K. ORMEROD1,2,5,4, 2Pruitt Family Dept. of Biomed. Engin., 3Depts. of Pediatrics and Neurol., 4Dept. of Neurosci., 5McKnight Brain Inst., 6Depts. of Pharmacol. and Therapeut., 7NF/SG VHS Med. Ctr., 1Univ. of Florida, Gainesville, FL

Abstract: There are approximately 2.2 million persons with epilepsy in the US. Temporal lobe epilepsy (TLE) is the most common epilepsy with over 25% of individuals resistant to antiepileptic drug treatment. Amygdala electrical kindling is a model of TLE that is used to identify the mechanisms of seizure evolution and maintenance and to test treatments. Kindling can increase the production of new cells that differentiate into granule neurons up to 8-fold in the hippocampus of adult rats. We have previously shown that somatostatin (SST) overexpression in the hippocampus prevents the development of severe seizure behavior in 70% of kindled rats and may even reverse kindling-induced seizure behavior along with the associated cognitive deficits. Male Sprague Dawley rats (225-250g) were implanted bilaterally into the amygdala with stimulating and recording electrodes and either sham kindled (n=5) or kindled (n=20) 2x per day (6h apart) until 3 consecutive grade 5 seizures were exhibited in the kindled rats. Some kindled rats received bilateral hippocampal injections (4µl each) of recombinant pAAV-CBa-GFP (n=4) or pAAV-CBa-SST-GFP (n=10) 48h after the last seizure. Three weeks later, rats were kindled every 48h for 3 weeks and seizure behavior was recorded. Rats were injected i.p. with the cell synthesis marker bromodeoxyuridine (BrdU; 100mg/kg) 48h after the final kindling session and perfused 4h later. Hippocampal sections were stained immunohistochemically to estimate total IBA+ microglia and dividing BrdU+ progenitor cell numbers stereologically and confirm resting, CD11B+ activated or CD68+ phagocytic IBA+ microglial and GFAP+/Sox2+ Type 1 or GFAP- /Sox2+ Type 2 BrdU+ progenitor cell phenotypes under confocal microscopy. Relative to sham-kindled rats, significantly more dividing BrdU+ progenitor cells were found in the hippocampi of kindled rats (p < 0.001) and kindled rats injected with a control GFP vector (p < 0.01) but not in kindled rats injected with a SST gene vector. Kindling upregulated proliferating Type 1 (p < 0.05) but not neuronally-committed Type 2a progenitor cells. Kindling did not alter the total number of microglia in the granule layer of the dentate gyrus but preliminary data suggest that a greater proportion of microglia were activated in the hippocampi of kindled rats. Data collected thus far support the hypothesis that kindling upregulates the division of naïve Type 1 neural progenitor cells and the number of activated microglia in the adult hippocampus and that SST gene therapy may protect neurogenesis and microglia from the effects of kindling and thereby reduce seizure severity.

Poster

495. *In vivo* and *In vitro* Models of Acute and Chronic Seizures

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 495.23/G37

Topic: C.07. Epilepsy

Title: *In vivo* evidence of GABA-A receptor-mediated inhibition restraint in acute seizure model


Abstract: Surround inhibition as a physiological defense mechanism against epileptiform activity remains poorly understood. We report an investigation of dual pharmacologically evoked seizure foci in an acute *in vivo* rat seizure model. One seizure focus was created by locally injecting 4-aminopyrine into somatosensory cortex. The focality and lack of propagation of this focus was verified using both a 96 channel microelectrode array arranged in a 10 x 10 grid with 400 um inter-electrode spacing implanted over the injection site, and *in vivo* calcium imaging. In order to focally disrupt the inhibitory restraint in a separate region, we injected bicuculline methiodide (a GABA-A receptor blocker) into another site 3-6 mm away to the 4-aminopyrine site. We found that this injection initiated seizure propagation and, in cases where bicuculine was injected distally from 4-aminopyrine focus, resulted in a new seizure focus that was non-contiguous to the 4-aminopyrine focus. Our analysis of temporal dynamics and spike timing demonstrated unit activity at the new noncontiguous focus exhibited opposed spike phase-locking and independent temporal dynamics. These experiments provide evidence that feedforward GABA-A synaptic conductance mediates an inhibitory restraint. Moreover, the effects of GABA-A synaptic conductance can occlude the presence of independent seizures arising from multiple noncontiguous sites in the low frequency local field potential. Furthermore, we found that ictal activity developed by interaction between the two sites can occur in the absence of pre-existing aberrant connectivity. Thus, this experiment counters the widely held hypothesis that a pre-existing, structurally abnormal “epileptogenic network” is necessary to...
incite multiple cross-site interactions in focal epilepsy. Dissecting these network factors may lead to more accurate seizure localization methods in the surgical treatment of epilepsy.


Poster

495. *In vivo* and *in vitro* Models of Acute and Chronic Seizures

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 495.24/G38

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH CounterACT R21NS084900

NYMC Intramural Grant

Title: A novel *in vitro* model for studying tetramethylenedisulfotetramine-induced neurotoxicity

Authors: *L. R. VOSE*¹, M. LAUKOVA², J. VELISKOA¹, L. VELISEK¹, M. P. SHAKARJIAN², P. K. STANTON¹; ¹Cell Biol. and Anat., ²Envrn. Sci., New York Med. Col., Valhalla, NY

Abstract: Tetramethylenedisulfotetramine (TMDT) is a hydrophilic neurotoxic compound mainly known for its use as a rodenticide. TMDT is highly potent, proconvulsant, odorless, colorless, and implicated in a significant number of accidental and intentional human poisonings, mostly in China. Despite bans worldwide, this compound is still illegally available in Chinese markets, making it a potential terrorism concern. Although TMDT is thought to act mainly as a GABAA receptor antagonist, exposure remains lethal to mice despite diazepam-induced anticonvulsant effects. Our recent studies report reduced lethality in TMDT-exposed mice treated with N-methyl-D-aspartate (NMDA) receptor antagonists, alone or in combination with benzodiazepines that enhance GABAA receptor activation. Brain inflammatory markers are elevated in rats exposed to TMDT and calcium influx is increased in cultured hippocampal neurons. Together, these data suggest that delayed neuronal death may be an ultimate outcome of TMDT exposure. Our objective was to establish organotypic hippocampal slice cultures as an *in vitro* model of TMDT toxicity for moderate-throughput screening of compounds that would minimize delayed neuronal death. In this model, organotypic slices were prepared from P8-P12 rats and, after a 1 week recovery period, incubated with varying concentrations of TMDT with or without drug treatment. After TMDT exposure, neuronal death was quantified by measuring...
propidium iodide and activated caspase fluorescence in the CA1, CA3, and DG pyramidal cell layers of the hippocampal slices. We found that TMDT had minimal effect on cell viability after 2 hours, but produced significant neuronal death after a 24 hour exposure. Additionally, low concentrations of TMDT markedly enhanced delayed neuronal death elicited by the glutamatergic agonist NMDA, even after 2h exposure. Neuronal death was reduced by treating cultures with MK-801, an NMDA receptor open channel blocker, or diazepam, an allosteric enhancer of GABAA receptors. Taken together, our data suggest that the organotypic slice culture model recapitulates features of TMDT poisoning in vivo, making it a useful model for elucidating molecular mechanisms underlying TMDT-induced neuronal death, and screening potential neuroprotective compounds. Understanding the pathways involved in TMDT-induced neurotoxicity and identifying compounds to minimize cell death will be critical to improving long-term neurological outcomes for individuals exposed to TMDT, goals of potential importance to national security. [Supported by NIH CounterACT R21NS084900 and NYMC Intramural Research Support Grant to MPS]


Poster

495. In vivo and In vitro Models of Acute and Chronic Seizures

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 495.25/G39

Topic: C.07. Epilepsy

Support: CounterACT Inter-Agency Agreement between NIH/NINDS(Y1-O6-9613-01) and USAMRICD (A120-B.P2009-2)

Title: Development and validation of a rat model of delayed treatment for nerve agent intoxication


Abstract: Organophosphate nerve agents act by covalently binding to, and inhibiting the activity of, acetylcholinesterase. The resultant increase in acetylcholine at neuromuscular junctions and within the central nervous system causes dramatic systemic and neurological

Deleted: in vivo

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effects in exposed individuals, including loss of muscle control, respiratory distress, status epilepticus, and often death. When administered within minutes of exposure, current therapies targeting the cholinergic and GABAergic systems can be extremely effective; however, awareness of a chemical threat and access to medical treatment is rarely so immediate. This is especially true in cases of widespread civilian exposure such as the attacks in Syria in 2013. Moreover, standard medical countermeasures lose efficacy against nerve agent-induced seizures because of recruitment of a variety of neurotransmitter systems and brain regions to the seizure generation and propagation process. An animal model of delayed treatment of central nervous system symptoms is critical for the development of more effective therapies. Here we describe a model in which adult male rats were exposed to a seizure-inducing dose of the nerve agent soman, began to show electroencephalographic evidence of seizures, and were then administered standard medical countermeasures (atropine sulfate, pralidoxime [2-PAM], and midazolam) at delayed time points after seizure onset. We then confirmed that this model is appropriate for identifying additive anticonvulsant and/or neuroprotective effects of potential test treatments by accompanying standard medical countermeasures with scopolamine, which has previously been shown to reduce seizure duration and associated neuropathology. We observed a dose-dependent increase in the termination of seizures as evidenced by a reduction in overall electroencephalographic power and specifically power in the gamma (20-70 Hz) range within one hour of scopolamine treatment. Similarly, the number of dying neurons quantified by FluoroJade B staining in the piriform cortex, the hippocampus, the amygdala, the thalamus, and the parietal cortex 24 hours after scopolamine treatment was reduced compared to controls. Current efforts using this model are focused on evaluating novel compounds solicited by the National Institute of Neurological Disorders and Stroke’s CounterACT Neurotherapeutics Screening Program.


Poster

495. In vivo and In vitro Models of Acute and Chronic Seizures

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 495.26/G40

Topic: C.07. Epilepsy

Support: Fapesp grant 2012/24282-2
Title: Transcriptome and proteome profile of dorsal and ventral dentate gyrus of a rat epilepsy model induced by electrical stimulation that presents classical hippocampal sclerosis

Authors: *A. S. VIEIRA*\(^1\), A. M. CANTO\(^2\), A. H. B. MATOS\(^2\), C. S. ROCHA\(^2\), B. CARVALHO\(^3\), V. PASCOAL\(^3\), R. GLIOLI\(^4\), I. LOPES-CENDES\(^2\); \(^2\)The Brazilian Institute Of Neuroscience And Neurotechnology (Brainn), \(^1\)Univ. Estadual De Campinas - Fcm, Campinas, Brazil; \(^3\)Depo De Ciências Básicas – Fe, Campus Universitário De Nova Friburgo, Univ. Federal Fluminense, Nova Friburgo, Brazil; \(^4\)Laboratory Of Animal Quality Control (Cemib), Univ. Estadual De Campinas, Campinas, Brazil

Abstract: An animal model based on eight hours of electrical stimulation of the perforant pathway (pp) in wake rats is capable of inducing hippocampal damage that more closely resemble that found in patients with mesial temporal lobe epilepsy. Furthermore, these animals develop spontaneous seizures after a latent period, however the molecular mechanisms involved in the induction of epileptogenesis remain unknown. RNAseq-based transcriptome analyzes and shotgun mass-spectrometry based proteomics offers the possibility of accurate profiling of global gene and protein expression. Therefore, the present study explores the molecular mechanisms responsible for epileptogenesis in this animal model using transcriptomics and proteomics tools. Electrodes were implanted bilaterally in the dentate gyrus (DG) and in PP of control (n=4) and experimental rats (n=4). After one week the PP of experimental rats were electrically stimulated for 8 hours after a protocol of two days pre-conditioning by PP electrical stimulation. Fifteen days following stimulation rats were euthanized and the brains processed for laser microdissected using Zeiss PALM LCM. Dorsal (dDG) and Ventral DG (vDG) were collected from each rat, total RNA and proteins were extracted, and libraries for RNAseq in Illumina Hiseq platform were prepared. Proteins were digested with trypsin and peptide fragments were analyzed in an HPLC coupled LTQ-Orbitrap Velos mass spectrometer. A total of 2,367 and 1,889 genes were found to be differentially expressed (p<0,05) when comparing the control and stimulated dDG and vDG respectively. Furthermore, a total of 42 and 50 proteins were found differentially expressed when comparing the control and stimulated dDG and vDG respectively. Gene ontology analysis indicated a predominance of inflammation related genes up-regulated in both dDG and vDG. Exclusively in the vDG there was a significant enrichment of axonal guidance and calcium signaling gene onthologies. It is noteworthy the presence of various differentially regulated genes involved in axonal guidance, synaptic function, neural electrical activity and neuropeptides. Moreover, different members of these families of molecules are exclusively differentially regulated in the dDG and vDG. The transcriptome and proteomics data explored in this study indicate many possible components of the molecular mechanisms responsible for epileptogenesis in an animal model that displays hippocampus sclerosis. Furthermore, even though similar mechanisms may be found in different DG sub-regions, the components involved in such processes seem to be region specific.

Poster

495. *In vivo* and *In vitro* Models of Acute and Chronic Seizures

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 495.27/G41

Topic: C.07. Epilepsy

Support: NIH Grant R01GM100768

Title: A *Drosophila* model of neonatal epileptic encephalopathy

Authors: W. CHI1, M. ALBERSEN2, Q. YANG1, M. BOSMA2, S. TURKSON1, N. M. VERHOEVEN-DUIF2, *X. ZHUANG1;

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Abstract: Pyridox(am)ine 5′-phosphate oxidase (PNPO) is a rate-limiting enzyme in converting inactive vitamin B6 (VB6) from the diet to pyridoxal 5′-phosphate (PLP), the only active form of VB6. PLP is a co-factor for more than 140 enzymes required for amino acid metabolism, gluconeogenesis and neurotransmitter (e.g. serotonin and GABA) synthesis. In humans, autosomal recessive PNPO deficiency leads to neonatal epileptic encephalopathy (NEE). NEE is characterized by severe seizures in newborns. They usually appear within hours of birth and are unresponsive to commonly used anticonvulsant drugs. Untreated, most patients die within weeks. New mutations in human PNPO (hPNPO) have been increasingly identified in NEE patients, yet the underlying causes of seizures and lethality are not understood. We have previously identified a c.95C > A missense mutation in SGLL – a homolog of hPNPO in *Drosophila melanogaster* and have shown that flies that carry the c.95C>A mutation (sgll95 flies) exhibited a lethal phenotype when fed on sugar-only diet. Here we report that the lethal phenotype of sgll95 flies is associated with very low PLP levels, and that lethality can be rescued by either wild-type (wt) SGLL (*drosophila* PNPO (dPNPO)) or wt hPNPO, but not hPNPOR95H, a mutant PNPO identified in NEE patients. In addition, sgll95 flies exhibit a seizure-like phenotype, which can also be rescued by wt dPNPO or wt hPNPO, suggesting the functional conservation of PNPO between humans and flies. Furthermore, both lethality and seizures are observed in global sgll knockdown flies, but the phenotypes are more pronounced than that in sgll95 flies. We are using tissue specific- and cell type specific- Gal4 drivers to determine the cell groups and neurotransmitter systems responsible for NEE-like phenotype. These *Drosophila* models...
represent promising approaches to study the pathogenesis of NEE caused by PNPO deficiency and to functionally characterize hPNPO mutations identified in human patients.


Poster

495. In vivo and In vitro Models of Acute and Chronic Seizures

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 495.28/G42

Topic: C.07. Epilepsy

Support: CONACYT (scholarship 326059 to EVC)

Title: Dose-response curve for pentylentetrazol-induced convulsions in developing rats

Authors: *E. VELAZCO*, I. ZAMORA*, A. A. PUIG*, R. A. MEDEL, M. L. LOPEZ-MERAZ;

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Abstract: Pentylentetrazol (PTZ) is a GABAA receptor antagonist and is widely used as a seizure model. Effects of PTZ in adult rats are well known; however, seizures induced by PTZ in developing rats have not been fully described. The goal of this study was to characterize seizures induced by different doses of PTZ in fourteen days-old (P14) rat pups. Wistar rats (both genders) were injected intraperitoneally with PTZ (Sigma) at doses of 45 (n = 4), 50 (n = 4), 55 (n = 10), 60 (n = 8), 65 (n = 8), 70 (n = 4) or 75 mg/kg (n = 4). The occurrence and latency to behavioral convulsion, as well as mortality rate was recorded for each experimental group. Data were analyzed using a proportion test or a Kruskall Wallis ANOVA. Results showed that 45 mg/kg PTZ caused myoclonic jerks but not generalized clonic-tonic seizures (GCTS). Twenty five percent of rats receiving 50 mg/kg PTZ had GCTS that eventually progressed to status epilepticus (SE). Rats injected with 55-75 mg/kg PTZ had GCTS (88-100%) and some of them developed SE (25-60%); however no differences in the proportion of rats displaying seizures were detected between the doses of PTZ tested (p>0.5). During SE, seizures were characterized by loss of righting reflex, swimming-like movements of forelimbs and hindlimbs and tremors. Mortality due to the GCTS was smaller in rats injected with 55 mg/kg PTZ when compared with 75 mg/kg PTZ (p=0.03). No difference in the latency to the CTGS was observed between the experimental groups (p>0.5). Our findings suggest that PTZ produces GCTS that evolve to SE at
doses from 55 mg/kg in P14 rat pups. An acute administration of PTZ could be a useful model to study SE in developing rats.


Poster

495. **In vivo and In vitro Models of Acute and Chronic Seizures**

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 495.29/G43

Topic: C.07. Epilepsy

Support: VIEP-BUAP/Health 2015 grants to JRE and CC

CONACYT grant 243247 to JRE

CONACYT grant 243333 to CC

Dr. Ygnacio Martínez, Vice-rector of Research

Title: The absence seizures in the myelin mutant taiep rat are sexually- and circadian- dependent

Authors: *M. CORTES*¹, Y. SILVA², J. R. EGUIBAR²;

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Abstract: The myelin mutant taiep rats have a progressive motor syndrome characterized by tremor, ataxia, immobility episodes (IEs), epilepsy and paralysis. It showed an initial hypomyelination followed by a progressive demyelination in the central nervous system. Continuous electroencephalographic (EEG) recordings in taiep rats showed a spike-wave discharge (SWD) in cerebral cortices and hippocampus similar to that reported in WAG/Rij and GAERS two well know absence epilepsy models. The aim of this study was to analyze the SWD in adult male and female taiep rats at 3 months of age. The rats were maintained under standard conditions with free access to rodent pellets and water. Using stereotaxic surgery three electrodes were implanted in the frontal, parietal and occipital cortices to record the electrocorticogram, two electrodes on the neck muscles for the electromyogram and one on the left extraocular muscles for electrooculogram, and bipolar electrode in the hippocampus to register the theta rhythm Our results showed higher incidence of SWD in the dark respect to light phase in female but not in male rats, (P<0.05). Mean duration of SWD in male taiep rats did not differ between light and
dark phase on the other hand female taiep rats mean duration is longer in the dark respect light phase (P<0.05). In conclusion, absence seizures is a sexual dimorphic behavior and also dependent of the light/dark phase suggesting that thalamo-cortical circuit that is modulated by steroid milieu as well as circadian cycle.

Disclosures: M. Cortes: None. Y. Silva: None. J.R. Eguibar: None.

Poster

495. In vivo and In vitro Models of Acute and Chronic Seizures

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 495.30/G44

Topic: C.07. Epilepsy

Support: FP7 CIG 303797

Title: Abnormal steady-state visual responses in a Drosophila model of epilepsy

Authors: *S. A. ALAMRI1,2, A. WADE1,2, C. ELLIOTT2,3; 1Psychology Dept., 2Biol. Dept., 3Univ. of York, York, United Kingdom

Abstract: Recent work using steady-state visually-evoked potentials (SSVEPs) has demonstrated abnormal visual gain control in human epilepsy patients (Porciatti et al 2000; Tsai et al 2011). In Drosophila, a mutation in the kcc gene (kccDHS1) renders young flies susceptible to light and shock-induced seizures (Hekmat-Scafe et al 2006) and has been used as a model of human juvenile epilepsy. Here, we used SSVEPs to study the age profile of contrast-driven photoreceptor and neuronal responses in both kccDHS1 and wild type (wt) Drosophila. To characterize gain control in these animals further, we also made measurements of contrast adaptation and spatial summation in the wild type and kccDHS1 visual system.

Methods: Full field luminance flicker sequences generated by a high intensity LED were modulated at linear combinations of 12Hz and 15Hz about a mean level. Stimulus-locked responses were measured using the electroretinogram (ERG) (Afsari et al 2014). We verified that the 1st and 2nd harmonics of the input frequencies originated in the photoreceptors and neural synapses respectively using histamine gated chloride channel mutants (ort) that eliminate neuronal signaling from the photoreceptors (Pantazis et al 2008). We measured contrast vs response functions from groups of at least 10 kccDHS1 and wt flies aged 1, 2, 3, 7, 10, 20 and 30 days as well as measures of contrast adaptation and spatial summation in 1 day old animals.
Results: The wild type photoreceptor responses were relatively constant across age groups while response from kccDHS1 flies increased with age and peaked around d30. Young kccDHS1 flies exhibited large, noisy neural responses compared to wt animals and a relative absence of gain control. kccDHS1 flies also exhibited strong but transient oscillations after stimulus onset (at around 80Hz) and offset (around 20Hz). Responses from kccDHS1 animals became more similar to wt flies by d20 - corresponding to a decrease in susceptibility to seizure. Finally, young kccDHS1 flies showed extremely high levels of contrast adaptation and reduced spatial tuning which normalized in older animals. Conclusion: SSVEPs can be used to measure both photoreceptor and neuronal responses in Drosophila. SSVEP responses from flies exhibit similar characteristics to those from other animals and can be used to measure neuronal gain control in wt flies as well as genetic models of neurological disease. In kccDHS1 flies, increases in response amplitude and variability, adaptation and susceptibility to seizures are correlated. This technique has the potential to identify reliable, in vivo biomarkers of epilepsy in invertebrate models of neurological disease.

Disclosures: S.A. Alamri: None. A. Wade: None. C. Elliott: None.

Poster
496. Antiseizure Therapies

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 496.01/H1

Topic: C.07. Epilepsy

Support: NIH/NINDS R01NS073768

Title: The role of regulatory T cells in the modulation of heightened neuronal excitability in epilepsy

Authors: D. XU¹, S. D. MILLER¹, *S. KOH²;
¹Dept. of Microbiology-Immunology and Interdepartmental Immunobiology, Feinberg Sch. of Medicine, Northwestern Univ., Chicago, IL; ²Pediatrics, Northwestern University, Feinberg Sch. of Med., Chicago, IL

Abstract: Rationale: Recurrent seizures activate immune responses can create a deleterious positive feedback loop. We have detected brain-infiltrating innate and adaptive immune cells in the resected brain of patients with intractable epilepsy and mice with recurrent status epilepticus (SE). In experimental autoimmune encephalomyelitis (EAE), we have demonstrated supplementation of regulatory T cells (Tregs) could reduce the severity of both active and
adoptive transfer EAE. We aim to identify the role of Tregs in the modulation of neuronal excitability and epileptogenesis. Methods: C57Bl/6-FoxP3-eGFP transgenic or wt C57Bl/6 mice were injected with kainic acid (KA) or PBS (control) at post-natal day (P)14 to induce SE (1st hit). Mice received a 2nd KA at P28 and were perfused 7 days later. Cerebral cortex was harvested, processed to generate single cell suspension and stained with fluorescently labeled cell markers for immune cells for flow cytometry. Intracellular cytokine staining (ICS) was performed on the brain-infiltrating leukocytes in vitro. Results: We used FoxP3-eGFP transgenic mice to study the role of Tregs in the two-hit model of epilepsy. Brain-infiltrating Tregs were significantly increased in mice after two-hit of KA-SE compared to single-hit control. A boost in the Treg frequency suggested a compensatory role of these immune-suppressive T cells in mice that experienced early-life seizures. We then examined Tregs in the spleen, as well as the cytokine secretion profiles of the antigen presenting cells and CD3+ T lymphocytes in mice treated with immune-modulating nanoparticles. There were more Tregs that secreted anti-inflammatory IL-10 cytokine and upregulated programmed death-1 (PD-1) in mice that had received nanoparticles. A corresponding decrease in the ability to secret pro-inflammatory cytokines was observed in the CD4+ and CD8+ T cell compartment. The proliferative capacity of the total CD3+ T cells was also affected in the nanoparticle-treated mice. Conclusions: Our preliminary data demonstrated that the control of peripheral leukocytes infiltration into the brain of mice with SE can be achieved by upregulating Treg frequency. Manipulation of the regulatory T cell compartment in conjunction with augmenting anti-inflammatory cytokine in the brain can potentially be used therapeutically to reduce immune-mediated neuronal excitability and epileptogenesis. It may further promote repair cascade. Whether early intervention in brain-specific inflammation can reduce detrimental consequence of SE remains to be determined.


Poster

496. Antiseizure Therapies

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 496.02/H2

Topic: C.07. Epilepsy

Title: Neuromodulation induced by low-intensity direct current stimulation: impact on interictal epileptic discharges

Authors: *F. WENDLING, F. MINA, G. DIEUSET, P. BENQUET; Inserm U1099, Rennes, France
Abstract: Most patients suffering from mesial temporal lobe epilepsy (mTLE) are drug-resistant. In the seizure onset zone, abnormal hyperexcitability of epileptic tissue, due to long lasting unbalanced ratio between potentiated glutamatergic excitation vs decreased GABAergic inhibition, triggers spontaneous recurrent seizures. In the case where surgery is not indicated, alternative therapeutic approaches are required. In vitro animal studies have established that stimulation-induced uniform weak electric fields modulate neuronal activity in brain slices. Overall, direct current stimulation (DCS) induces short-lasting effects (through immediate effects on neuron membrane potential) followed by longer-lasting aftereffects (through modulation of long term plasticity of glutamatergic synapses). Therefore DCS might be used to reduce epileptic activity. To date, only few experiments were performed in vivo and still, evidence must be brought that in vitro results can be translated in vivo, in particular in freely moving epileptic animals with chronically implanted electrodes. One study reported a significant increase in the afterdischarge threshold in amygdala-kindled rats, based on the daily application of direct currents, at 5-15 µA intensity during 15 min (Weiss et al., 1998). This consideration led us to investigate the effects of invasive very low intensity Local Direct Current Stimulation (LDCS) applied for short periods in kainate-treated mice experiencing frequent hippocampal paroxysmal discharges (HPDs). Using a combined computational/experimental approach, we first investigated the effects of low intensity LDCS on HPDs in a computational model accounting for stimulation-induced neuron membrane polarization. We then tested the model predictions in vivo and analyzed some safety issues like stimulation-induced heating and metal deposition (redox reactions) in the brain tissue. Our computational study shows that HPDs can be significantly reduced by cathodal DC stimulation. This prediction was confirmed in freely moving epileptic mice with no major thermal or histological damage. We observed beneficial effects on the occurrence of epileptic activity for very low stimulation intensities (1 µA) and for short duration current applications (30s). In these conditions, stimulation effects were reversible and histological damage was not obvious. Nevertheless, iron deposit was detected in the stimulated hippocampus indicating the occurrence of redox reactions due to stimulation. Novel stimulation protocols are currently being investigated to overcome this limitation.

Disclosures: F. Wendling: None. F. Mina: None. G. Dieuset: None. P. Benquet: None.

Poster

496. Antiseizure Therapies

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 496.03/H3
**Topic:** C.07. Epilepsy

**Support:** NIH Grant F31NS086429

NIH Grant NS35915

CURE Taking Flight Award

Epilepsy Foundation

**Title:** Application of closed-loop optogenetics to uncover the role of hippocampal dentate gyrus microcircuits in temporal lobe epilepsy

**Authors:** *A. BUI¹, E. KROOK-MAGNUSON², C. ARMSTRONG¹, S. LEW¹, M. OIJALA¹, I. SOLTESZ¹;

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**Abstract:** Temporal lobe epilepsy is the most common type of epilepsy in adults, but unfortunately, in over one-third of patients, seizures are not controlled with currently available treatment options. Understanding of the microcircuits alterations underlying seizure activity is a key step in the development of improved therapeutical approaches. One brain region that has been of intense interest is the hippocampal dentate gyrus, partly due to the fact that the dentate gyrus undergoes hallmark alterations in association with temporal lobe epilepsy, including hilar cell loss and mossy fiber sprouting. In chronically epileptic mice, using *in vivo* electrophysiological and closed-loop optogenetic methods, we are able to modulate specific populations of cells in the dentate gyrus immediately upon detection of spontaneous behavioral and electrographic seizures. With this approach, we have found that through optogenetic manipulation of excitatory populations of cells in the dentate gyrus, we are able to inhibit or amplify seizure activity through recruitment of different microcircuits. We showed that targeting either the granule cell circuitry or the sparse population of mossy cells can alter seizure dynamics. This study demonstrates the importance of the dentate gyrus circuit in the propagation of seizures, and as a promising target in the control of temporal lobe seizures.

**Disclosures:** A. Bui: None. E. Krook-Magnuson: None. C. Armstrong: None. S. Lew: None. M. Oijala: None. I. Soltesz: None.

**Poster**

496. Antiseizure Therapies

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM
Title: Intracerebroventricular transplantation of human fetal brain-derived neural stem/progenitor cells restrains seizures in the lithium-pilocarpine model of rat temporal lobe epilepsy

Authors: *H. Lee¹, S. Yun¹, I.-S. Kim², I.-S. Lee³, J. Shin³, K. Park²,¹
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Abstract: Temporal lobe epilepsy (TLE), the most common and intractable type of adult focal epilepsy, is typically associated with pathological alterations in the hippocampus and parahippocampal regions. TLE is an attractive target for cell therapy due to its focal nature and associated cellular defects. Neural stem/progenitor cells (NSPCs) can continuously self-renew and give rise to intermediate and mature cells of both neuronal and glial lineages. Following transplantation in the diseased brain, NSPCs exhibit the potential to migrate toward the lesion and replace degenerated or ablated cells, as well as deliver therapeutic substances. In this study, we transplanted human NSPCs (hNSPCs), derived from an aborted fetal telencephalon at 13 weeks of gestation and expanded in culture as neurospheres over a long time period, into the lateral ventricles of lithium-pilocarpine induced epileptic rats. Implanted hNSPCs migrated and integrated into the recipient brain. The majority of hNSPCs remained undifferentiated, although subsets of donor-derived cells differentiated into all three neural cell types of the central nervous system and expressed inhibitory neurotransmitter gamma-aminobutyric acid (GABA). We found that hNSPC transplantation significantly reduced the frequency and duration of spontaneous recurrent motor seizures (SRMS) at 2 and 3 months post-transplants. In addition, hNSPC-transplanted epileptic rats showed neuroprotection, restoration of astrocytic glial cell-derived neurotrophic factor (GDNF) expression, and up-regulation of anti-inflammatory cytokines in the hippocampus. Finally, we demonstrated that conditioned medium from hNSPCs has neuroprotective action in an *in vitro* model of glutamate excitotoxicity. These results suggest that hNSPC transplantation possesses a therapeutic potential for treating TLE.

Poster

496. Antiseizure Therapies

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 496.05/H5

Topic: C.07. Epilepsy

Support: NS079977

Title: Neuronal mechanisms of the antiepileptic effects of human pluripotent stem cell-derived maturing GABAergic interneurons

Authors: *J.-H. CHO¹, M. CUNNINGHAM², S. CHUNG²;
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Abstract: Seizure disorders debilitate more than 65,000,000 people worldwide, with temporal lobe epilepsy (TLE) being the most common form. Previous studies have shown that transplantation of GABA-releasing cells results in suppression of seizures in epileptic mice. Derivation of interneurons from human pluripotent stem cells (hPSCs) has been reported, pointing to clinical translation of quality-controlled human cell sources that can enhance inhibitory drive and restore host circuitry. We have previously demonstrated that hPSC-derived maturing GABAergic interneurons (mGINs) migrate extensively within the epileptic hippocampus and reduce seizure activity and other behavioral abnormalities in a mouse model of TLE. In this study, we used electrophysiological approaches to investigate the neuronal mechanisms of the antiepileptic effects of transplanted human mGIN. Although they showed immature passive membrane properties, approximately half of transplanted human mGINs fired spontaneous action potentials, indicating that they are tonically active even without extrinsic synaptic inputs. Moreover, transplanted human mGINs fully integrated into the hippocampal circuitry, receiving excitatory synaptic inputs from host glutamatergic neurons, and were therefore activated by host glutamatergic neuron. In turn, our optogenetic studies revealed that grafted human mGINs release inhibitory neurotransmitter GABA in an activity-dependent manner. Therefore, the activation of transplanted mGINs, either by spontaneous activity or by excitatory synaptic drive, can cause an increase of inhibitory synaptic responses in host hippocampal neurons, shifting excitation/inhibition balance toward inhibition and suppressing exaggerated neural activity in the epileptic brain. Our studies suggest that grafted human mGIN could reduce seizure activity by regulating inhibitory balance in the epileptic hippocampus.

Disclosures: J. Cho: None. M. Cunningham: None. S. Chung: None.
**Title:** Neurogenic stem cell behavior in models of epilepsy with and without brain damage

**Authors:** T. WILSON, G. KIM, *K. W. THOMPSON; Biol., Occidental Col., Los Angeles, CA

**Abstract:** Cell-based therapy has been proposed for intractable temporal lobe epilepsy. Targeting potentially therapeutic cells to the seizure-prone hippocampus has shown promise in both seizure reduction and neural repair/replacement. Several studies have suggested that embryonic stem cell-derived neural cells (ESC-NS) will have clinical utility because they can differentiate and integrate into the host brain. Objective: Here we investigated transplantation strategies with an ESC-NS line, which has been engineered to become neurogenic, in models of temporal lobe epilepsy. Methods: Neurogenic ESC-NS were transplanted into the hippocampi of rats that had either established seizures with brain damage (pilocarpine-treated (N=8)), or without brain damage (fully kindled (N=4)), or in animals prior to kindling-induced seizures (N=5), or in controls without damage (naïve N=3)) or controls with damage (6-OHDA-treated N=2)). To track the cell survival and distribution, weeks after transplantation, we used nissl stains, X-gal histochemistry, and immunohistochemistry for β-galactosidase. Results: We found that the pattern of stem cell dispersion, and the interaction of stem cells with resident hippocampal cells, is dependent on the model used. In a model that has both hippocampal damage and established seizures, stem cell markers were found distal to the transplantation site and in cells with morphologies consistent with mature hippocampal neurons. This pattern of staining was not seen in animals with established seizures but no damage, nor in animals transplanted prior to hippocampal seizure induction, nor in controls. Conclusions: We have shown here that the microenvironment produced by seizure-induced cell degeneration, but not seizures alone, participates in stem cell behavior after transplantation. These data are consistent with earlier studies that have shown that transplanted cells distribute uniquely in different models of disease. However, recent reports showing that ESC-NS have the capacity to fuse with host neurons raise caution around interpreting these data as purely cell replacement.

Poster

496. Antiseizure Therapies

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 496.07/H7

Topic: C.07. Epilepsy

Support: CONACYT-CB-2009-01 Grant #130194 for Ureña-Guerrero, M.E.

Title: GABAA receptor activation in newborn female rats increases the expression level of several GABAergic markers in the hippocampus and entorhinal cortex, and diminishes seizure susceptibility

Authors: *M. E. URENA-GUERRERO, K. FLORES-HUITRADO, J. MURGÚIA-CASTILLO, C. BEAS-ZÁRATE, A. FERIA-VELASCO; Univ. De Guadalajara (CUCBA), Zapopan, Jalisco, Mexico

Abstract: High seizure susceptibility observed in neonates has been related to the immaturity of nervous system, which is characterized by a predominant excitatory activity linked to neuronal differentiation processes. Therefore, in early developmental stages GABA through GABAA receptor (GABAA-R), produces neuronal depolarization contributing to excitation, with the development the GABA effect changes to neuronal hyperpolarization and contributes to inhibition. The functional GABA change is related to a developmental regulated shift on chloride (Cl-) gradient. At neuronal level, NKCC1 acting as Cl- importer is highly expressed when the GABA induces neuronal depolarization, whereas KCC2 acting as Cl- exporter increases its expression in neurons to reach the establishment of GABA-mediated inhibition. In vitro studies have suggested that in early developmental stages, GABA-mediated neuronal excitation increases the KCC2 expression improving the neuronal inhibitory response to the GABA application. In this work, in vivo GABAA-R activation was induced in newborn female Wistar rats through the subcutaneous (s.c.) administration of muscimol (1 mg/kg of body weight) at postnatal day (PD) 0. After the treatment, at PD 15, the expression levels of KCC2, and alpha1 and gamma2 GABAA-R subunits were estimated in the hippocampus and entorhinal cortex using western-blotting assays. Furthermore, susceptibility to convulsive activity induced by 4-aminopyridine (4-AP; 2 mg/kg of body weight by s.c. administration) was behaviorally evaluated. Results indicate that muscimol treatment induced a significant increment in the expression level of all proteins in both cerebral studied regions, in at least two fold higher respects to control group. The treatment also reduced the susceptibility to 4-AP, increasing the
latency for the appearance of generalized tonic-clonic convulsions (GTCC), reducing the maximum number of GTCC episodes and the duration of convulsive activity. In previous work carried out in males, muscimol treatment had similar effects to those reported here. This suggests that an activation of GABA-A-R in early developmental stages, when the excitatory activity of GABA is predominant, may improve the GABAergic inhibitory signaling and reduce the seizure susceptibility in both genders.


Poster

496. Antiseizure Therapies

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 496.08/H8

Topic: C.07. Epilepsy

Support: Conacyt 239594

PAPIIT IN211913

Title: Time-restricted feeding inhibits seizure susceptibility in a pharmacological seizure model by means of metabolic and epigenetic changes

Authors: *J. LANDGRAVE-GÓMEZ*1,2, O. MERCADO-GOMEZ2, M. VAZQUEZ-GARCIA2, V. RODRIGUEZ-MOLINA2, R. GUEVARA-GUZMAN2;

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Abstract: Introduction: Although new generation of antiepileptic drugs have emerged lately, approximately one-third of epilepsy patients do not respond to classical pharmacological treatments. Objectives: We investigated whether time restricted feeding (TRF) is able to ameliorate seizure susceptibility and if it involves associated-metabolism and epigenetic modifications. Methodology: The acute seizure model in rats consisted in a pre-treatment of lithium chloride (3 mEq/kg) followed by pilocarpine administration (60 mg/kg) with a previous injection of scopolamine nitrate (1 mg/Kg) 30 min before. Briefly TRF was to allow rats to feed for two hours daily during their light phase for 21 days; control and pilocarpine animals consisted feeding ad libitum. Biochemical changes were measured in each group along the 21 days and 24 h after drugs administration. Results: We found that TRF inhibits seizure
susceptibility including a prolonged latency to the first seizure; a decrease in seizure severity and fewer animals with status epilepticus. In addition, TRF showed a significant reduction of power of seizures compared with animals fed ad libitum (AL) as shown in electrophysiology measures. In order to understand which mechanisms may be involved on TRF beneficial effects we measured several physiological and metabolic parameters such as phosphorylation of kinases involved in regulation of metabolic pathways (AMPK and Akt); body weight, food intake, blood glucose and β-hydroxybutyrate (β-HB) concentration in blood. We found that TRF induces a general metabolic shift towards catabolic pathways; inducing an increase of phosphorylation on AMPK and decreasing the phosphorylation on Akt kinase in hippocampus and liver and therefore increasing the concentration of β-HB in blood. In addition, we also found a significant increase in acetylation of H3K9 and H3K14 in the hippocampus of this group compared with AL. Conclusion: Our data demonstrate that TRF is capable to diminish seizure susceptibility. This phenomenon may be mediated by chromatin remodeling of genes by means of inhibition of HDACs by β-HB of associated genes involved in generation of epileptic seizures. The first author received a fellowship from Conacyt. This project was supported by PAPIIT IN211913 and Conacyt 239594 grants.


Poster

496. Antiseizure Therapies

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 496.09/H9

Topic: C.07. Epilepsy

Support: NIMH Grant MH071739

NINDS Grant NS074785

NINDS Grant NS024067

Title: Lysophosphatidylinositol (LPI), an agonist of the noncanonical cannabinoid receptor GPR55, increases excitatory neurotransmitter release and reduces inhibitory synaptic strength

Authors: *E. C. ROSENBERG*1,2, O. DEVINSKY3,1, R. W. TSIEN1,2;

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Abstract: Cannabis has been used as a treatment for seizures since ancient times. Two of the major phytocannabinoids in the cannabis plant are the psychoactive compound Δ9-tetrahydrocannabinol (THC) and its non-psychoactive counterpart cannabidiol (CBD). Recent studies indicate that CBD may prevent seizures and reduce mortality in epileptic animal models with low toxicity and high tolerability. While the exact mechanism of CBD is not well understood, new research indicates that CBD acts as an antagonist at the orphan G-protein coupled receptor, GPR55. When activated by the endogenous ligand L-α-lysophosphatidylinositol (LPI), GPR55 causes a transient increase in intracellular Ca²⁺ and vesicular release probability at excitatory hippocampal synapses (Sylantyev et al. 2013). CBD opposes this effect, suggesting a potential antiseizure action. In contrast to these clear findings at glutamatergic synapses, the role of GPR55 at inhibitory synapses remains unknown. In this study, we characterized the distribution and function of GPR55 at inhibitory hippocampal synapses in vitro. Results from immunocytochemistry suggest that GPR55 was localized in presynaptic excitatory terminals labeled with VGLUT to a greater degree than inhibitory terminals labeled with GAD65. Postsynaptically, GPR55 localized to somas and dendrites of CaMKII (+) pyramidal neurons, parvalbumin (PV+) interneurons, and somatostatin (SST+) interneurons. Furthermore, using whole cell patch clamp electrophysiology of hippocampal pyramidal neurons, we found that application of LPI increased the frequency of mEPSCs with no change in amplitude. Conversely, LPI application reduced the amplitude of mIPSCs with minimal change in frequency. Preliminary results suggest that LPI acts through GPR55 to reduce GABAₐ,R expression, possibly mediated by a decrease in GABAₐ,R 3 Ser 408/409 phosphorylation. Taken together, these results suggest that LPI increases the excitatory / inhibitory ratio in hippocampal neuronal networks by a dual mechanism: enhancing excitatory transmission and attenuating inhibition. This predicts that CBD, by opposing LPI action, may exert its beneficial anti-seizure effects on both excitatory and inhibitory synapses.

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Poster

496. Antiseizure Therapies

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 496.10/H10

Topic: C.07. Epilepsy

Support: NIH Grant 1 R21 TW009384-01
Title: Responsive transcranial focal electrical stimulation via tripolar concentric ring electrodes delayed the development of electrical amygdaloid kindling in the cat

Authors: *A. VALDÉS-CRUZ*, W. G. BESIO, B. VILLASANA-SALAZAR, V. M. MAGDALENO-MADRIGAL, D. MARTÍNEZ-VARGAS, S. ALMAZÁN-ALVARADO, R. FERNÁNDEZ-MAS

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Abstract: Noninvasive transcranial focal electrical stimulation (TFS) is an experimental proposal to treat pharmacoresent epilepsy. Our group investigated the effects of TFS on amygdaloid kindling (AK) epileptogenesis in cats. In initial experiments, 40 minutes application of subthreshold TFS through a tripolar concentric ring electrode (TCRE) (10 mm of diameter) placed on vertex, prior to kindling was not effective. Nevertheless, we found a reduction in spectral power after secondarily generalized seizures. These results suggest that TFS is not innocuous. Hence, the present study aimed to analyze the effect of TFS via CRE applied responsively on seizure activity induced by AK in freely moving cats. Nine adult cats were tested. Stainless steel bipolar electrodes were stereotaxically implanted into both temporal lobe amygdalae (AP: 11.5, L: 9.5, H: 5.0) and prefrontal cortices. In addition, a TCRE was fixed on the skull at the temporal bone bilaterally (P: 11.5, L: 15.0). Cats were assigned to two experimental groups: control AK group and AK-TFS group. In the first group, daily AK (1 second train, 1 ms pulses, 60 Hz, 300-600 µA) was performed until all animals reached kindling stage VI (tonic-clonic generalized seizures). In the AK-TFS group, AK was applied every 24 h, TFS through a TCRE placed in the temporal bone was delivered for 2 minutes (biphasic square pulses, 300 Hz, 200 µs, 2.5 mA) ipsilaterally and AK at the same time for 40 days. After, only AK was applied until animals reached kindling stage VI. The experimental variables quantified were the amygdaloid afterdischarge duration and frequency, the number of AK trials to reach kindling stage VI and the maintenance in kindling stages. We found a delay in the progression within behavioral stages of kindling and that animals remained in stage II (partial seizures) during the days of TFS. The number of stimulations to reach stage VI in control AK animals was 29.0 ± 4.0, and animals of AK-TFS stimulation showed a significant increase (84.33 ± 7.33, p< 0.001). In addition, behavioral development was retarded, with an increased number of stimulations required to reach stage III (control AK 9.66 ± 7.33 vs AK-TFS 51.33 ± 10.33, p< 0.001). In AK-TFS group, overall kindling development was delayed and amygdaloid afterdischarge duration and frequency of spikes did not showed a progressive increase as was observed in the control AK group. Our results suggest that TFS applied responsively interferes with the development of convulsive evolution and secondary generalization. This delay effect may be due to the interruption of abnormal EEG activity and the interference of the propagation pathways.
**Disclosures:**  
A. Valdés-Cruz: None.  
W.G. Besio: None.  
B. Villasana-Salazar: None.  
V.M. Magdaleno-Madrigal: None.  
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**Poster**

496. Antiseizure Therapies

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#: 496.11/H11**

**Topic:** C.07. Epilepsy

**Support:** HHMI-CURE Medical Research Fellowship

- NIH Grant R01 NS066974
- NIH Grant R21 NS083783

**Title:** Dual-site pontine and thalamic neurostimulation to restore consciousness during and after seizure

**Authors:**  
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**Abstract:** In cases of medically or surgically refractory epilepsy, impaired consciousness during and following seizures has a dramatic impact on quality of life, morbidity and mortality. Improving consciousness in the ictal and postictal periods would be highly beneficial to patients. Our lab developed a rodent model of partial limbic seizures which mimics the human cortical electroencephalographic signature of neocortical slow waves and behavioral arrest associated with loss of consciousness in temporal lobe epilepsy. We additionally demonstrated EEG suppression of the arousal system, including the brainstem cholinergic, and intralaminar thalamic nuclei. Here we investigated the effects of combined intralaminar thalamic and pontine stimulation on cortical arousal in the rodent limbic seizure model. We targeted electrodes to the intralaminar central lateral thalamus (CL) and pontine nucleus oralis (PnO), and confirmed localization with histology. Seizures were induced by brief 2 second hippocampal stimulation at 60 Hz. We then stimulated bilateral intralaminar thalamic CL at 100 Hz and PnO at 50 Hz at varying current intensities during seizures for 120 seconds while synchronously recording electrophysiology and behavior. Single site stimulation of CL alone or PnO alone was insufficient to produce reliable cortical desynchronization and behavioral improvement during
seizures (n=12). In contrast, combined CL and PnO stimulation during seizures reduced cortical slow waves by more than 85% while simultaneously eliciting robust behavioral arousal as measured by spontaneous exploratory behavior (n=6). Effects of PnO and CL stimulation to reduce cortical slow waves and increase behavioral arousal resembled those seen with stimulation during physiological sleep (n=6) and under anesthesia (n=6). These data suggest a novel potential therapeutic approach for improving consciousness during the ictal and postictal states. If paired with responsive neurostimulation algorithms, it may lead to rapid implementation of a therapy for preventing impaired consciousness during and after seizures in epilepsy patients. Further work is needed to determine the degree of recovery in the ictal and postictal periods based on explicit behavioral tasks. Multi-site stimulation within the arousal networks appears to be a potent therapeutic approach which may also benefit other states of decreased consciousness such as vegetative and minimally conscious states.


Poster

496. Antiseizure Therapies

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 496.12/H12

Topic: C07. Epilepsy

Support: INP Grant 123240.1

Title: Effect of vagus nerve stimulation on PTZ-induced seizures in rats with thalamic reticular nucleus lesion

Authors: *E. VELÁZQUEZ-MIRANDA*¹, R. D. CONTRERAS-LÓPEZ², S. ALMAZÁN-ALVARADO³, R. FERNÁNDEZ-MAS², V. M. MAGDALENO-MADRIGAL²;
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Abstract: Vagus nerve stimulation (VNS) in experimental models of epilepsy has demonstrated to have anticonvulsive effects. However, the mechanisms through which it exerts these effects are still unknown. It has been suggested that the thalamic reticular nucleus (TRN), through the cortico-thalamic circuit could be participating in the anticonvulsive effect. The aim of this study was to analyze the effect of VNS on convulsive and non-convulsive seizures induced by pentilenetetrazole (PTZ). Wistar rats (300g) were used and divided into five groups: 1) TRN
lesion VNS group, which underwent lesion with radiofrequency in the TRN (AP -1.44, L 2.0, DV 6.2) and received VNS through a bipolar electrode designed in our laboratory that was implanted into the left vagus nerve, caudal to the larynx; 2) TRN sham VNS group, in which electrodes were introduced into the TRN, but no lesion was performed, and received VNS; 3) TRN lesion group, which received TRN lesion with radiofrequency but no VNS; 4) VNS group, which in contrast, received VNS but no TRN lesion; and 5) Control group, which received no manipulation other than the implantation of epidural stainless screws in the frontal, parietal and occipital cortices. All animals, with the exception of the Control group, were implanted with stainless steel bipolar electrodes into the hippocampus (AP -3.6, L 2.0, DV 3.5) and the posterior nucleus of the thalamus (AP -3.6, L 2.0, DV 5.4) and with epidural stainless screws in the frontal and parietal bones. VNS was administered continuously for 60 minutes or duty cycle (1 min on/ 5 min off). To observe effects on both convulsive and non-convulsive seizures, PTZ was administered at low doses (10 mg/kg) every 15 minutes until the first generalized tonic clonic seizure (GTCS) was reached. Variables quantified were latency to reach GTCS, GTCS duration and number of PTZ doses needed to reach GTCS. We observed that both the lesion and the sham-lesion in the TRN reduced latency and number of PTZ doses necessary to reach a GTCS. Also, the TRN lesion group had longer GTCS in duration than the Control group. Our results suggest that TRN integrity is not necessary for the anticonvulsive effect of VNS. On the other hand, manipulating the TRN, in some cases with or without lesion, could be disrupting an important inhibitory thalamo-cortical pathway and this disruption facilitates seizure generalization.


Abstract: Significant limitations are associated with studying seizures induced by acute exposure to convulsants in otherwise normal in vitro and in vivo preparations. Traumatic brain injury is a major cause of medically intractable acquired epilepsy. The slicing preparation of organotypic hippocampal slice cultures parallels traumatic axonal shear injury and subsequently slices develop a dense recurrent connectivity that results in the development of spontaneous seizures after 1 week in vitro. We utilized this in vitro model of severe post-traumatic epilepsy with a reproducible, accessible and accelerated course of epileptogenesis to conduct a blind screen of over 500 drug-concentration combinations for anticonvulsant, antiepileptic and neuroprotective effects in chronic epilepsy. Lactate and LDH levels were assayed in spent culture media as biomarkers of seizure activity and ictal neuronal death, respectively, with the latter being correlated with seizure burden. Compounds exhibiting significant anticonvulsant activity in chronic-application screens advanced to a second stage consisting of wash-out screens to differentiate anticonvulsant from antiepileptogenic effects as well as in vitro electrophysiological confirmation. Anticonvulsant/proconvulsant and neuroprotective/neurotoxic effects were expressed as ratios of lactate production and LDH release in the presence of drug vs. control conditions. These effects were normally distributed about a zero mean effect. Both distributions exhibited a small skew toward therapeutic efficacy, with approximately 5% of all tested drug-concentration combinations demonstrating therapeutic effects on seizure activity and/or cell death rates that were > 3 standard deviations from the mean. These in vitro assays for anticonvulsant and neuroprotective effects in a model of severe pediatric post-traumatic epilepsy demonstrated that, just as in human trials, clinically-available anticonvulsants had modest antiepileptic effects. Several compounds demonstrated both anticonvulsant and neuroprotective effects that we interpret to represent reductions in seizure activity and consequent ictal cell death. The third stage was comprised of double-blind, crossover-controlled, in vivo EEG testing in the kainate model of chronic epilepsy to confirm the anticonvulsant effect of a lead compound, celecoxib, a cyclooxygenase-2 inhibitor. This technology comprises a promising strategy for the rapid, staged investigation of drug efficacy in pediatric post-traumatic epileptogenesis, and could be further scaled with available robotic technologies.


Poster

497. Anticonvulsant Pharmacological Therapies
**Title:** Neuronal sodium elevation and COX-2 activation in post-traumatic epileptogenesis *in vivo*

**Authors:** *T. BALENA, Y. SAPONJIAN, K.-J. PARK, K. J. STALEY;*  
Massachusetts Gen. Hosp., Charlestown, MA

**Abstract:** Post-traumatic increases in intracellular Cl\(^-\) can result in GABA becoming depolarizing, which could lead to disinhibition and early post-traumatic epileptic seizures. Increases in intracellular anions are likely to be balanced by an increase in intracellular cations, and the consequent salt accumulation could underlie cytotoxic edema. We investigated post-traumatic changes in intracellular Na\(^+\) concentration ([Na\(^+\)]\(_i\)) using organotypic hippocampal slice cultures from wild-type C57BL/6J mice, imaged with the Na\(^+\) dye SBFI. Two-photon imaging was used to excite SBFI at both Na\(^+\)-sensitive and -insensitive wavelengths, allowing for the ratiometric determination of the [Na\(^+\)]\(_i\). Hippocampal neurons exhibited a broad distribution of [Na\(^+\)]\(_i\), and in many cases [Na\(^+\)]\(_i\) was significantly higher than has been reported in undamaged neurons. All values of [Na\(^+\)]\(_i\) were stable for hours. Neurons with highest values of [Na\(^+\)]\(_i\) were more likely to stain for propidium iodide, suggesting that [Na\(^+\)]\(_i\) elevation is an early and possibly progressive biomarker of eventual neuronal death. Population studies indicated that high [Na\(^+\)]\(_i\) values were more common immediately after slicing trauma, and returned to low levels within ~2 days. At longer incubation times, during which slices become epileptic, [Na\(^+\)]\(_i\) again became elevated, returning to physiological levels at 20 DIV, at which age seizure intensity diminished. Acute perfusion of 10 µM of the Na\(^+\)/K\(^+\) ATPase inhibitor ouabain or 100 µM of the KCC2/NKCC1 antagonist furosemide increased [Na\(^+\)]\(_i\). 10 µM of the NKCC1 antagonist bumetanide or 100 µM of the Na\(^+\)/Ca\(^{2+}\) exchange antagonist benzamil also increased [Na\(^+\)]\(_i\), indicating that in the days after trauma NKCC1 and the Na\(^+\)/Ca\(^{2+}\) exchanger operate in the reverse of their canonical directions by exporting Na\(^+\) and, presumably, the cotransported ions. Application of 8 µM fluorescein conjugated to large dextran molecules indicated severe membrane disruption in only a small number of neurons, but a moderate amount of damage in a larger number of neurons, particularly as epileptogenesis progresses. Perfusion of 10 µM of the selective non-steroidal anti-inflammatory drug celecoxib significantly reduced [Na\(^+\)]\(_i\) in nearly all neurons, and also caused significant reductions in the power, frequency, and duration of...
seizures. Overall, elevated \([\text{Na}^+]\), is a promising new biomarker for compromise of neuronal membrane permeability, which precedes many traditional indicators of epileptic activity and ictal cell death, and the ability of celecoxib to mitigate elevated \([\text{Na}^+]\), thus represents a prime therapeutic target for the early treatment of epilepsy.

**Disclosures:**  
T. Balena: None. Y. Saponjian: None. K. Park: None. K.J. Staley: None.

**Poster**

**497. Anticonvulsant Pharmacological Therapies**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 497.03/H15

**Topic:** C.07. Epilepsy

**Support:** NIH Grant NS 40109-13  
NIH Grant NS 34700-21

**Title:** Pharmacological targeting of the WNK-SPAK kinase complex to modulate neuronal Cl-homeostasis and cell volume in recurrent seizures

**Authors:**  
*V. I. DZHALA*¹, Y. SAPONJIAN¹, K. KAHLE², K. STALEY¹;  

**Abstract:** Neuronal chloride concentration ([Cl]ᵢ) is an important determinant of both post-synaptic GABAₐ-receptor mediated signaling and cell volume regulation. Restoration of [Cl]ᵢ equilibrium after synaptic activity is achieved by the net regulated activities of the cation-Cl⁻ co-transporters (CCC) NKCC1 (mediating Cl⁻ influx) and KCC2 (mediating Cl⁻ efflux). Hypoxia-ischemia, acute brain trauma, and recurrent seizures not only alter the equilibrium value of Cl⁻ but also impair the functional regulation of the CCCs. The end result is cell swelling, acute and chronic accumulation of [Cl]ᵢ, and GABA depolarizing responses, which foster seizures, epileptogenesis and anticonvulsant resistance via failure of inhibition. Antagonizing NKCC1 activity and/or stimulating KCC2 activity might reduce swelling and [Cl]ᵢ in injured neurons, restore GABAergic inhibition and suppress seizures. NKCC1 and KCC2 are stimulated and inhibited, respectively, by direct phosphorylation mediated by the Cl⁻-sensitive WNK (lysine-deficient protein kinase)-activated SPAK (proline/alanine-rich kinase) kinase. We therefore speculated that WNK-SPAK inhibition might be an especially potent strategy to promote neuronal Cl⁻ extrusion by coincident NKCC1 inhibition and KCC2 activation. We determined
the acute and chronic anticonvulsive and neuro-protective efficacy of STOCK1S-50699, a recently-identified compound that disrupts the WNK-SPAK interaction, antagonizes WNK signaling, and thereby inhibits NKCC1 and KCC2 phosphorylation. Organotypic hippocampal slice cultures from mice expressing the Cl− sensitive fluorescent protein Clomeleon or Super Clomeleon were studied as a model of acute and chronic traumatic brain injury. After a 1-week latent period, slice cultures developed spontaneous seizure activity. We found that: STOCK1S (i) reduced the frequency and power of early recurrent seizures by 65% and 68% respectively; (ii) the anti-convulsive effect was irreversible and coincident with cytopathological deterioration suggestive of neurotoxicity; (iii) induced bi-directional effects on neuronal chloride transients and cell volume in different sub-populations of neurons; (iv) strongly reduced the amount of early and late seizure activity as assayed by lactate production; (v) strongly increased the amount of early LDH production indicating a severe neurotoxic effect. Our results demonstrate that STOCK1S, the only available inhibitor of WNK-SPAK signaling, significantly reduces seizure frequency and power but exhibits neurotoxicity, highlighting the need for novel non-toxic strategies of targeting the WNK-SPAK pathway for therapeutic benefit.


Poster

497. Anticonvulsant Pharmacological Therapies

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 497.04/H16

Topic: C.07. Epilepsy

Title: An *in vitro* epileptogenesis mouse model for anticonvulsant drug screening

Authors: *C. EHNERT, A. GRAMOWKI-VOSS, B. M. BADER, O. H.-U. SCHROEDER; Neuroproof Gmbh, Rostock, Germany

Abstract: Epilepsy is characterized by spontaneous, recurrent seizures. A seizure is seen in an EEG by high excessive and synchronous activity and can be correlated with synchronized and high frequency firing of neuronal populations in the CNS. Here, we present a novel potential cell culture assay with the spontaneous occurrence of ictal activity episodes. We developed a culture protocol using primary hippocampal neurons from early embryonic mice, grown on microelectrode arrays (MEAs) and maintained for several weeks. The ictal activity is characterized by the appearance of sudden spontaneous synchronized bursts of action potentials with duration of at least 5 seconds. The activity patterns of the hippocampal network activity are recorded and analyzed by multivariate data analysis of 204 activity describing parameters using...
our in-house software NPwaveX. These parameters quantify the network activity changes regarding general activity, synchronicity, connectivity, oscillation, and burst structure. The assay presented here enables the study of neuronal networks and their respective activity that resemble epileptogenic brain cells in vivo. Acute treatment with antiseizure drugs affects the burst structure parameters such as burst duration. Interestingly only long bursts are affected. A major advantage over hippocampal slice preparations, these cultures allow chronic treatment with novel compounds over several weeks thereby allowing studying epileptogenesis. Thus, further developing and validation of this assay could enable the discovery innovative drugs with new mode of actions. Applying this assay to a 48-well or 96-well MEA platform the throughput for compound screening will increase, hence enhancing the drug development for anticonvulsant and anti-epileptogenic compounds.

Disclosures:  C. Ehnert: A. Employment/Salary (full or part-time); NeuroProof GmbH. A. Gramowski-Voss: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeuroProof GmbH. B.M. Bader: A. Employment/Salary (full or part-time); NeuroProof GmbH. O.H. Schroeder: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeuroProof GmbH.

Poster

497. Anticonvulsant Pharmacological Therapies

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 497.05/H17

Topic: C.07. Epilepsy

Support: NC3R CrackIT Neuratect

Title: Prediction of drug-induced seizure-liability in human iPSC-derived neuronal networks compared to primary mouse networks - functional, phenotypic in vitro assessment using micro-electrode arrays

Authors: *A. GRAMOWSKI-VOSS, A.-M. PIELKA, C. EHNERT, K. JUEGELT, O. H.-U. SCHROEDER, B. M. BADER; NeuroProof GmbH, Rostock, Germany

Abstract: Human stem cell (hiPSC)-based in vitro platforms are promising to serve as an alternative to animal in vivo or in situ models such as labor-intensive and time-consuming behavior tests or using brain slices. Testing new chemicals with neuroactive, neurotoxic, and
Seizurogenic effects stem cell-derived neuronal platforms will be physiologically relevant by incorporating the features of current neurotoxicity testing in animals and will further improve current state-of-art by offering higher throughput and higher content. Our aim was to increase biological complexity for an adequate phenotypic and functional read-out of human neuronal systems. Therefore we co-cultured commercially available hiPSC-derived neurons with glia cells or different neuronal populations in order to evaluate the functional effects induced by known seizurogenic compounds. We used multiwell microelectrode arrays (mwMEAs, Axion Biosystems, Inc.) for medium-throughput screening of electrophysiological maturation for several weeks in vitro. We tested known seizure-inducing compounds and excitatory/non-seizure-inducing compounds in concentration-response experiments which were analyzed by our in-house software NPwaveX computing 204 activity describing parameters from the spike train. With these compound specific activity profiles we developed a so called “seizure score” to evaluate drugs potential to induce seizurogenic activity. We compare the results from concentration-response experiments between human iPSC-derived neuronal networks and those of functionally mature primary mouse cortical networks. In summary, for the hiPSC-based model we increased biological and functional complexity by mixing glia and neurons and optimizing culture conditions to obtain relatively mature neuronal networks which are reactive to neurotoxic compounds. Based on the multi-parametric data, we computed a novel classifier “seizure score” which was calibrated by reference compounds. Thus, it is equivalent to our previously presented classifier based on primary mouse neuronal networks. In conclusion, our classification technology allows investigating and comparing the safety margin of novel drug candidates between rodent and human cell background and thereby dramatically increases the prediction of seizurogenic risks.


Poster

497. Anticonvulsant Pharmacological Therapies

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 497.06/H18

Topic: C.07. Epilepsy

Support: MCST R&I-2013-014

Title: Interaction between cannabinoid type 1 and serotonin 2C receptors in the pilocarpine model of status epilepticus in rat
**Authors:** *R. COLANGELI*¹, M. PIERUCCI¹, R. DI MAIO², G. DI GIOVANNI¹;
¹Physiol. and Biochem., Univ. of Malta, Msida, Malta; ²Pittsburgh Inst. for Neurodegenerative Dis. and Dept. of Neurology, Univ. of Pittsburgh, USA., Pittsburgh, PA

**Abstract:** Status epilepticus (SE) is a neurological disorder characterized by continuous or rapidly repeating seizures. Cannabinoid type 1 receptor (CB1R) regulates neuronal excitability and has been shown to mediate the anticonvulsant effects of cannabinoids in several animal models of epilepsy. Several studies support the existence of crosstalk mechanisms between endocannabinoid (EC) and serotonin (5-HT) systems. The 5-HT2C receptor (5-HT2CR) subtype has received attention in epilepsy as KO mice for this receptor displayed increased seizure susceptibility, whilst its activation showed anticonvulsant effects in different models of epilepsy. Interaction between 5-HT2CR and CB1R has been shown; for instance CB1R KO mice exhibit altered expression and impaired functionality of the 5-HT2CR in several brain areas. Here we tested the interaction between 5-HT2CR and CB1R in the prevention of SE using the rat pilocarpine (PILO) model. Adult male Sprague Dawley rats were injected with PILO (360 mg/kg) and monitored for 3 hours by cortical electroencephalographic (EEG) and hippocampal local field potential recording. Seizure behaviour was also observed and severity was measured by Racine scale. Pre-treatment with the cannabinoid agonist WIN 55,212-2 (WIN), the 5-HT2CR agonist RO 60-0175 (RO) or their combination (RO+WIN) was performed 45 min before PILO administration. PILO induced SE in 12/14 rats (Racine scale 4-5). Both WIN and RO, administered alone, had no effect in preventing EEG seizures. However, WIN administered alone reduced the severity of behavioural SE (Racine scale 2-3). RO+WIN administered in combination significantly reduced the occurrence of SE. Power spectrum analysis revealed that RO+WIN significantly reduced the total power during SE, in respect to the vehicle group. The effect of RO+WIN was completely blocked by the administration of CB1R antagonist AM 251. Intriguingly, administration of 5-HT2CR antagonist SB 242084 prior to RO+WIN treatment strongly potentiated the antiepileptic effect of RO+WIN since no animal displayed SE. This suggests that different 5-HT receptor subtypes or other unknown receptors might be involved in SE RO-induced effects. Data so far obtained indicate a synergistic interaction between the 5-HT system and CB1R in SE, although the exact role of the 5-HT2CR remains to be clarified. Our findings suggest that the cross-talk mechanisms between EC and 5-HT systems might represent a suitable target for the identification of new antiepileptic treatment of SE.

**Disclosures:** R. Colangeli: None. M. Pierucci: None. R. Di Maio: None. G. Di Giovanni: None.

**Poster**

497. Anticonvulsant Pharmacological Therapies

**Location:** Hall A
**Title:** Major impact of the first-line antiepileptic treatment choice on the second-line treatment efficacy in a mouse model of absence epilepsy

**Abstract:** Possible aggravation of epilepsy by antiepileptic drugs is an already known phenomenon. Overdoses and drug interactions are the two main reasons. However, seizures can also be worsened because of an inadequate treatment. This is often the case for children epilepsies such as childhood absence epilepsy. We addressed the problem of whether an inadequate first-line treatment could abolish the efficacy of a second-line treatment that would have been successful if applied as a first-line treatment. We used an inbred mouse model for absence epilepsy, BS/Orl, manifesting spontaneous and recurrent spike-wave discharges. Mice were submitted to an experimental protocol where they received two consecutive treatments. From the age of five weeks, mice were given valproate (VPA - reference), vigabatrin (VGB - known to aggravate the absence epilepsies) or ethosuximide (ESM - a specific for absence epilepsies) during 14 days. And then, they all received VPA during 42 days. A fourth group has received a saline solution (PHY) during the whole experiment. The 4 groups were assessed at 5 different times: before any treatment, after the first-line treatment and 3 times during the second-line treatment. After the first-line treatment, the 3 groups VPA, VGB and ESM were differing significantly as expected: compared to PHY, VGB was found to worse seizures whereas VPA and ESM were found to reduce seizures with a much greater effect for ESM. Interestingly, the application of the second treatment showed various effects. While the seizure level in the ESM group was much lower than in the VPA group after the first-line treatment, this benefit has progressively disappeared with the introduction of the VPA. Finally, after 6 weeks of VPA treatments, both ESM-VPA and VPA-VPA were presenting the same seizure occurrence rate. Conversely, while the VGB has aggravated the seizure level compared to the PHY group during the first-line treatment, the introduction of the VPA as the second treatment, has failed to reverse the tendency of an aggravation of the seizure level due to the initial application of the VGB. This study illustrates that an inadequate first-line treatment, more than worsening seizures, can have long-term adverse effects by reducing the efficacy of a posterior treatment.

Poster
497. Anticonvulsant Pharmacological Therapies

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 497.08/H20

Topic: C.07. Epilepsy

Support: DFG Grant FOR1103

Title: BUM13, a novel bumetanide derivative with reduced diuretic but enhanced anticonvulsant activity

Authors: *C. BRANDT¹, K. TÖLLNER¹, P. W. FEIT¹, M. GABRIEL², W. LÖSCHER¹, T. ERKER²;
¹Univ. of Vet. Medicine/Dept. of Pharmacol., Hannover, Germany; ²Dept. of Medicinal Chemistry., Univ. of Vienna, Vienna, Austria

Abstract: The cation-chloride cotransporters NKCC1 and KCC2 seem to be crucial in the pathogenesis of epilepsy. In this respect, the diuretic drug bumetanide, an inhibitor of NKCC1, has attracted interest because it represents a valuable tool for studying the role of intraneuronal chloride concentrations in epilepsy. Further, it represents a potential therapeutic strategy to prevent or treat epilepsy. However, two major drawbacks of bumetanide, its diuretic potential and low brain penetration, restrict the utility of bumetanide in experimental and clinical settings. The aim of this study was to characterize the pharmacokinetic and functional characteristics of the bumetanide derivative BUM13 [5-(anilinomethyl)-3-(butylamino)-2-phenoxy-benzenesulfonamide], which was selected from a large series of bumetanide analogues designed to be more lipophilic for improving brain penetration. Furthermore, BUM13 exhibited decreased affinity to NKCC2, which is mainly expressed in the kidney and is responsible for bumetanide’s diuretic potency. All investigations were performed in female NMRI mice. Bumetanide and BUM13 were injected intravenously at equimolar doses. For determining the diuretic effect, urine production was measured 15, 30, 60, 90, and 120 min after injection. Plasma and brain concentrations of bumetanide and BUM13 were analysed 30 and 60 min after injection using HPLC. The anticonvulsant effect of bumetanide and BUM13 were evaluated in the maximal electroshock threshold test (MEST) in naive vs. epileptic mice, because NKCC1 is higher expressed in the epileptic vs. the nonepileptic adult brain. Beginning six weeks after a pilocarpine induced status epilepticus (SE), bumetanide and BUM13 were tested alone or in
combination with phenobarbital (PB). Age-matched sham-treated non-epileptic mice served as control group. BUM13 was hardly metabolized to bumetanide. Brain penetration and brain/plasma ratio of BUM13 were much higher but the diuretic effect was significantly decreased compared to bumetanide. BUM13 itself did not have an anticonvulsant effect in the MEST, but in combination with PB, BUM13 increased the seizure threshold by 240% in epileptic mice, while PB was ineffective when injected alone. In nonepileptic control mice this effect of BUM13 was much less pronounced. Bumetanide alone did not exert an anticonvulsant effect nor did it enhance the effect of PB in the MEST. The results of this study indicate that BUM13 is an interesting alternative to bumetanide for the treatment of epilepsy. The mechanism of the marked potentiation of PB’s anticonvulsant activity needs to be further characterized, but inhibition of NKCC1 is a likely explanation.


Poster

497. Anticonvulsant Pharmacological Therapies

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 497.09/H21

Topic: C.07. Epilepsy

Support: NIH Grant K01NS069583

NIH Grant R01NS089698

Title: Effects of Calpain Inhibition on Epileptogenesis

Authors: *M. I. GONZALEZ, J. CARLSEN, P. LAM;
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Abstract: A characteristic of epilepsy is the appearance of unpredictable seizures arising from disordered and synchronous firing of neurons. In particular, temporal lobe epilepsy is a subtype of acquired epilepsy that often develops after stroke, traumatic brain injury or status epilepticus. Pathologic activation of calpain, a calcium dependent protease ubiquitously expressed in neurons, has been observed following experimental status epilepticus. Calpain activation after a brain injury triggers a series of neurotoxic signaling cascades that results on the cleavage of membrane receptors, cytoskeletal and structural proteins that negatively affect neuronal function and promote neuronal death. Unfortunately, the particular role of calpain overactivation on the
epileptogenic process is mostly unknown. This study is an initial evaluation of the possible role of calpain overactivation on epileptogenesis. Using the pilocarpine model of experimental epilepsy, we found that the time-course for the down-regulation of GABAergic proteins is similar to time-course of calpain overactivation. This suggested the possible role of calpain on the loss of inhibitory neurotransmission previously detected. In follow-up experiments, we found that pharmacological inhibition of calpain immediately after pilocarpine-induced status epilepticus delays epilepsy onset and reduces seizure burden. Ongoing studies in our laboratory are aimed to analyze the effects of calpain inhibition on additional hallmarks associated with epilepsy progression. A long-term goal of our studies is to generate a better understanding of the molecular events triggering epilepsy after a brain injury.

Disclosures: M.I. Gonzalez: None. J. Carlsen: None. P. Lam: None.

Poster
497. Anticonvulsant Pharmacological Therapies

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

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Topic: C.07. Epilepsy

Support: NIH R01 NS065957

FP7/2007-2013

n°602102 (EPITARGET)

Title: Glycine transporter 1 is a target for the treatment of epilepsy

Authors: *H. SHEN1, E. V. VLIET2, K.-A. BRIGHT3, M. HANTHORN1, N. LYTLE1, J. GORTER3, E. ARONICA2,3, D. BOISON1;

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Abstract: Glycine is the major inhibitory neurotransmitter in brainstem and spinal cord, whereas in hippocampus glycine exerts dual modulatory roles on strychnine-sensitive glycine receptors and on the strychnine-insensitive glycineB site of the N-methyl-D-aspartate receptor (NMDAR). In hippocampus, the synaptic availability of glycine is largely under control of glycine transporter 1 (GlyT1). Since epilepsy is a disorder of disrupted network homeostasis affecting
the equilibrium of various neurotransmitters and neuromodulators, we hypothesized that changes in hippocampal GlyT1 expression and resulting disruption of glycine homeostasis might be implicated in the pathophysiology of epilepsy. Using two different rodent models of temporal lobe epilepsy (TLE) - the intrahippocampal kainic acid model of TLE in mice, and the rat model of tetanic stimulation-induced TLE - we first demonstrated robust overexpression of GlyT1 in the hippocampal formation, suggesting dysfunctional glycine signaling in epilepsy. Overexpression of GlyT1 in the hippocampal formation was corroborated in human TLE samples by quantitative real time PCR. In support of a role of dysfunctional glycine signaling in the pathophysiology of epilepsy, both the genetic deletion of GlyT1 in hippocampus and the GlyT1 inhibitor LY2365109 increased seizure thresholds in mice. Importantly, chronic seizures in the mouse model of TLE were robustly suppressed by systemic administration of the GlyT1 inhibitor LY2365109. We conclude that GlyT1 overexpression in the epileptic brain constitutes a new target for therapeutic intervention, and that GlyT1 inhibitors constitute a new class of antiepileptic drugs. These findings are of translational value since GlyT1 inhibitors are already in clinical development to treat cognitive symptoms in schizophrenia.

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 cardiomyocytes of the rat. The aim of the present study was to determine if PPB modifies the electrographic activity and the subsequent neuronal damage in hippocampus when applied after 2 h of Status Epilepticus (SE) induced by pilocarpine. Male Wistar rats previously implanted with a bipolar electrode into the right ventral hippocampus received an administration of pilocarpine (300 mg/kg, i.p.) to induce SE. SE+DZP group (n=6) received an injection of diazepam (DZP 2.5 mg/kg, i.m.) 2 h after the SE establishment. The electrographic activity of hippocampus was analyzed using fast fourier transform method throughout all the experiment (up to 2 h after DZP). The SE+DZP+PPB group (n=6) was manipulated as described above, except that animals received PPB (178 mg/kg, i.p.) 1 h after DZP. One day after the SE, the animals were sacrificed and the brain used to evaluate the site of electrode implantation (Nissl staining), neuronal damage (FLUORO-JADE B) and neuronal preservation (NeuN immunofluorescence) in ventral hippocampus. In both experimental groups, the SE was established at 43.2±2.5 min after pilocarpine injection. The electrographic activity revealed faster, high-voltage rhythmic spikes and increase in spectral potency in beta, gamma and ripples bands during the SE. After DZP injection, the SE+DZP group showed a decrease in the seizure activity, an effect associated with decline in the potency of ripples oscillations (p<0.001). In contrast, after of PPB administration the SE+DZP+PPB group demonstrated lower electrographic activity that correlated with reduced potency in gamma and ripples bands (p<0.001). When compared with SE+DZP group, SE+DZP+PPB group demonstrated decreased neuronal damage in ventral hippocampus (dentate gyrus 468%, p<0.05, CA3 453%, p<0.001 and CA1 609%, p<0.001)) as well as an increase in NeuN immunopositive cells (CA3 377%, p<0.05 and CA1 353%, p<0.01). We conclude that the administration of PPB at the end of the SE reduces the epileptiform activity, an effect associated with a decrease in neuronal damage.


Poster

497. Anticonvulsant Pharmacological Therapies

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 497.12/H24
Title: Lactate dehydrogenase is a molecular target to regulate seizures

Authors: *N. SADA, T. INOUE;
Okayama Univ., Okayama, Japan

Abstract: Currently-available antiepileptic drugs are not effective for one third of patients with epilepsy. It is known that ketogenic diets are effective treatment for a part of the drug-resistant epileptic patients. Although ketogenic diets act on metabolic pathways, there are no antiepileptic drugs acting on metabolic pathways. In this study, we examined which metabolic molecules regulate membrane potentials in neurons and seizures in mice. Using slice patch-clamp techniques, we found that neurons were hyperpolarized by a switch from glucose to ketone bodies in ACSF. The hyperpolarization was recovered by an addition of lactate. Neurons were also hyperpolarized by inhibition of lactate dehydrogenase (LDH). We then examined effects of LDH inhibition on a chronic seizure model in mice, induced by intrahippocampal injection of kainate. In vivo recording revealed that LDH inhibition suppressed paroxysmal discharges in the seizure model. So far, there are no reports that LDH enzymes are inhibited by clinically-used antiepileptic drugs. We therefore explored LDH inhibitors from twenty clinically-used antiepileptic drugs, using an enzymatic assay. The enzymatic assay revealed that stiripentol, an antiepileptic drug for treatment of Dravet syndrome, was an LDH inhibitor. Furthermore, we found a stiripentol analog that inhibits LDH and strongly suppresses seizures in the mouse model. These results show that LDH enzyme could be a promising molecular target for antiepileptic drugs based on ketogenic diets.

Disclosures: N. Sada: None. T. Inoue: None.
Abstract: Introduction: Epilepsy is a common neurological disorder. Generalized epilepsies, which are characterized with fast neural discharges, lead to the myoclonic contractions and loss of consciousness. Antiepileptic drugs are ineffective in controlling seizures in about one-third of the patients. Therefore, drug resistant sufferers are in need for alternative treatment regimens. Flavonoids are suggested to possess potential benefits against epilepsy. In the present study, we investigated antiepileptic properties of rutin, a member of the flavonol subfamily.

Methods: A total of 40 female Wistar were assigned to five groups as Con. (received vehicle (DMSO); n=10), R10 (received 10 mg/kg rutin; n=10), R50 (received 50 mg/kg; n=10), and R100 (received 100 mg/kg; n=10). Picrotoxin was administered intraperitoneally to all animals in a dose of 2.5 mg/kg to startle seizures. A single dose of rutin, according to the belonging group, was injected intraperitoneally 30 mins before the picrotoxin administration. The epileptic activity was scored by an experienced observer.

Results: The administration of 10 and 50 mg/kg rutin prolongs the latency of the onset of seizure and decreases the total seizure count. The highest dose of rutin (100 mg/kg) shortens the total duration of seizure.

Discussion: According to our results, different doses of rutin exert diverse effects on the pathophysiological processes in the model of picrotoxin-induced epilepsy. Picrotoxin is an antagonist of GABA receptors and so, blocks Cl-channels. Decreased GABAergic and increased glutamatergic transmission are accused in epilepsy. Rutin seems to interact with these events to show an antiepileptic-like activity to some extent. Therefore, rutin may be considered as an adjuvant in drug resistant epilepsy.


Poster

497. Anticonvulsant Pharmacological Therapies

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 497.14/H26

Topic: C.07. Epilepsy

Support: Defense Threat Reduction Agency Joint Science and Technology Office, Medical S & T Division

Title: Diphenhydramine and latrepirdone effects on seizures and brain injury in rats following exposure to the chemical warfare nerve agent soman
Authors: *J. W. SKOVIRA, T. L. DAO, J. A. LEUSCHNER, R. K. KAN;

Abstract: Seizures complicate the treatment of casualties following nerve agent exposure and can lead to extensive brain damage and increased mortality if not controlled. Excessive cholinergic neurotransmission, predominately involving muscarinic receptors, is believed to initiate seizures following nerve agent exposure. Diphenhydramine is an antihistamine with potent antimuscarinic properties and has been shown to be a neuroprotectant. The present study was designed to evaluate two antihistamines, one with potent antimuscarinic effects (diphenhydramine) and a second with no antimuscarinic properties (latrepirdone) for their ability to prevent/terminate nerve agent-induced seizures and reduce neuropathology. Male Sprague-Dawley rats, weighing 250-300 g, were pretreated with HI-6 (125 mg/kg, ip) 30 min prior to GD administration (180 µg/kg, sc). One minute after GD challenge, animals were given atropine methyl nitrate (AMN; 2.0 mg/kg, im). Antihistamine treatments were given along with AMN or at the onset of seizures. The incidence of convulsions, percentage of mortality and extent of neuropathology were assessed. Diphenhydramine (40mg/kg) given as a treatment in combination with AMN one minute after GD challenge was effective in reducing the occurrence of convulsions, the incidence of mortality and the development of brain pathology in brain regions known to be vulnerable to GD-induced damage. When given after seizure onset diphenhydramine also significantly reduced the incidence of convulsions, improved mortality rate and prevented brain pathology. Latrepirdone (25mg/kg) failed to reduce the incidence of convulsions, mortality, and brain pathology following GD exposure. These observations suggest that diphenhydramine is effective in preventing GD-induced seizures, death and brain pathology. Further investigation of antihistamine drugs with anticholinergic properties for use as medical countermeasures to nerve agent poisoning is warranted.


Poster

497. Anticonvulsant Pharmacological Therapies

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 497.15/H27

Topic: C.07. Epilepsy

Title: Efficacy of a second generation neuroactive steroid, SAGE-217, in a mouse model of chronic medial temporal lobe epilepsy
Authors: R. S. HAMMOND, *G. M. BELFORT, A. J. ROBICHAUD, J. J. DOHERTY; Sage Therapeut., Cambridge, MA

Abstract: Neuroactive steroids are a class of endogenous and synthetic compounds that potentiate both synaptic and extra-synaptic GABA_A receptors. SAGE-217 is a potent and efficacious Second Generation neuroactive steroid GABA_A receptor positive allosteric modulator with anti-convulsant activity in rodent pentylentetrazole, 6 Hz and lithium-pilocarpine seizure models (Robichaud et al, 2015). Here we further characterized the anticonvulsant activity of SAGE-217 in the mouse kainate model of chronic mesial temporal lobe epilepsy (MTLE), a condition which affects an estimated 10% of the total epileptic population. Intraperitoneal injection of SAGE-217 at 1, 3 and 5mg/kg significantly suppressed the number and cumulated duration of hippocampal paroxysmal discharges (HPDs) in a dose dependent manner. A greater than 90% reduction in HPDs was obtained with 3 and 5mg/kg of SAGE-217 between 10 and 70 minutes post-injection and with the 5 mg/kg dose over the entire two hour recording period. In_vivo activity at the GABA_A receptor at concentrations determined in brain samples from satellite animals correlated with efficacy against HPDs. The capacity to reduce HPDs was benchmarked against other antiepileptic drugs. The activity of SAGE-217 at 3 and 5 mg/kg was superior to the effect of diazepam (2mg/kg). This study provides the first evidence of neurosteroid efficacy in the chronic kainate MTLE model and supports developing compounds of this class as novel antiepileptic drugs.

Disclosures: R.S. Hammond: A. Employment/Salary (full or part-time); SAGE Therapeutics. G.M. Belfort: 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.
Authors: *R. K. GOEL*¹, N. K. BHANGU²;
¹Professor,, Patiala, India; ²Dept. of Pharmaceut. Sci. and Drug Res., Punjabi Univ., Patiala, India

Abstract: Purpose: Resistance to epileptogenesis is common in different animal models of epilepsy. It was hypothesized that comparative analysis of neurochemical status of brains of resistant and sensitive animals may be useful find treatment approaches for pharmacoresistant epilepsy Method: Chemical kindling in male swiss albino mice was induced by giving a subconvulsant dose of 35 mg/kg PTZ intraperitoneally (i.p.), every alternate day for 68 days. Animals were observed for 30 min, and seizure activity scored according to a slightly modified Racine scale. Successfully kindled and resistant animals were divide into groups (n=10) respectively, while another group of naïve animals served as control group. After 2 hours of last pentylenetetrazole administration, all animals were sacrificed to remove their brains. Neurochemical [monoamines (norepinephrine, dopamine and serotonin), amino acids (glutamate, serine, arginine, glycine, taurine, alanine, GABA) and their metabolites (5-HIAA, DOPAC, Kynurenine)] changes were estimated using HPLC-ECD method, in discrete brain parts. Additionally, acetylcholinesterase activity and total nitrite levels were also estimated using microtiter reader method. PTZ brain and serum levels were also estimated. All experiments were approved by the IAEC of Punjabi University Patiala, India, (Approval No. 107/99/CPCSEA-2013-27) Results: The results of the neurochemical estimation demonstrated the remarkable changes in monoamines, amino acids and their metabolites, also elevated nitrosative and acetylcholinesterase activity in the kindled animals in comparison to resistant or naïve animals. Interpretation of these results suggested significant shift of 5HTP metabolic pathway to kynurine and subsequently to quinolinic acid responsible for induction of kindling in sensitive animals, whereas, its shift was observed towards serotonin in resistant animals. Conclusion: The present findings suggest that modulation of central 5HTP pathway may be one of the strategies for treatment of pharmacoresistant epilepsy. Ongoing studies are evaluating this approach in Lamotrigine kindling model of pharmacoresistance.


Poster

497. Anticonvulsant Pharmacological Therapies

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 497.17/H29

Topic: C.07. Epilepsy
Support: CONACYT Grant 106179

Title: Effect of spartein on the Status Epilepticus induced in rats by Pentylenetetrazole, Kainic Acid and Pilocarpine

Authors: *F. V. VILLALPANDO VARGAS, L. MEDINA-CEJA;
Biologia Celular y Mol., Univ. De Guadalajara, Guadalajara, Mexico

Abstract: The status epilepticus (SE) is defined as the prolonged seizure or recurrent seizures without full recovery between them, lasting longer than 30 min. Globally, the SE occurs one million per year, with a mortality rate of at least 30% in developing countries. The sparteine (Sp) is a quinolizidine alkaloid (QA) synthesized from most of Lupine species; the anticonvulsive effect of Sp was evaluated in the PTZ model of SE in which the Sp delayed the onset of convulsive behavior and increased the survival period. But there are not posterior efforts to determine clearly the anticonvulsive effect of Sp. For this reason we consider important to study the anticonvulsant effects of Sp at behavioral level and EEG activity in three different SE models. The convulsive behavior and survival period were analyzed 30 min after Sp administration (13mg/kg, i.p.) as well as the amplitude, frequency, duration and latency of epileptiform activity. The results showed normal behavior in control animals (saline solution, SS 0.9% and Sp administration) but in animals treated with only pentylentetrazole (PTZ, 90 mg/kg, i.p.; n=8), the convulsive behavior was progressive according Velisek scale (phase I-V), when the animals were pretreated with Sp a delayed in loss of animal posture was observed as well as an increase in latency to reach the scales III to V. While, animals with kainic acid (KA, 9mg/kg, i.p.; n=6) or pilocarpine (370 mg/kg, n=6) had a progressive convulsive behavior reaching the highest level in Racine scale (5); the animals treated with Sp before KA had a significant decrease in duration of seizures and an increase in latency to reach the scale 1 to 3. Animals treated with Sp before pilocarpine had an increase in survival. The EEG analysis showed slow activity in control animals (SS or Sp) as well as in the basal recordings of all the animals (before drug administration). The PTZ group presented rhythmic spike-waves and discharge trains of high amplitude and frequency. Animals with Sp before PTZ showed rhythmic spike-waves and discharge trains of low amplitude and frequency. While animals with KA showed an epileptiform pattern characterized by polyspikes and spike-wave, the pretreatment with Sp decreases the amplitude and frequency of epileptiform activity. The group with pilocarpina showed rhythmic spike-waves and discharge trains of high amplitude and frequency; the pretreatment with Sp decreases the amplitude and frequency of epileptiform activity. In conclusion, the anticonvulsant effect of the pretreatment of Sp 30 min before PTZ, AK and pilocarpine was observed in convulsive behavior (decrease of severity) and epileptiform activity (decrease in amplitude and frequency).

Disclosures: F.V. Villalpando Vargas: None. L. Medina-Ceja: None.
Antioxidant effects of tridecanoin, the triglyceride of decanoate, appear to contribute to its anticonvulsant effects

Authors: *K. TAN*¹, C. CARRASCO-POZO¹,², K. BORGES¹;
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Abstract: The medium-chain triglyceride (MCT) ketogenic diet has been proven effective in the treatment of pediatric epilepsy. Although ketone bodies, which increase significantly in the plasma of patients following the treatment, are thought to be the main contributors of the anticonvulsant effects, the correlations between ketone body levels and seizure control are yet to be established. Therefore, we investigated the anticonvulsant effects of two main components of the MCT ketogenic diet, triglycerides of eight-carbon octanoic acid and ten-carbon decanoic acid in acute mouse seizure models. We found that when given as 35% of total calories to male CD1 mice for ten days, tridecanoin but not trioctanoin was anticonvulsant in 6 Hz seizure model, increased latency to reach the first generalised seizures in fluorothyl model and improved the survival of mice in the pilocarpine model. The anticonvulsant effects appeared to be independent of the levels of ketone body β-hydroxybutyrate in the brain and plasma. We observed an increase in antioxidant power in the plasma and mRNA levels of heme oxygenase 1 and forkhead box O1 in the hippocampal formations of mice fed tridecanoin diet, suggestive of antioxidant effects of tridecanoin. Mitochondrial functions in cultured astrocytes were also assessed following a two-hour incubation with 200 µM of octanoic or decanoic acid in the form of oxygen consumption rate. Both octanoic and decanoic acids increased the basal respiration and ATP turnover but only decanoic acid resulted in an increase in proton leak of the mitochondria in comparison to 1 mM of sodium pyruvate. Taken together, the antioxidant effects and improvement of mitochondrial functions by decanoic acid may contribute to its anticonvulsant effects.

Disclosures: K. Tan: None. C. Carrasco-Pozo: None. K. Borges: None.
497. Anticonvulsant Pharmacological Therapies

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 497.19/H31

Topic: C.07. Epilepsy

Title: Mechanistic approach to neuroprotective potential of Lacosamide in seizures

Authors: *B. KUMAR¹, B. MEDHI²;
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Abstract: Epilepsy is a chronic neurological condition characterized by recurrent seizures, almost affects people in every country throughout the World. Recently, the neuroscientists and neurologists have investigated the use of antiepileptic drugs to prevent neuronal loss and alter the cognitive impairment commonly seen with the progression of epilepsy. In this study, we evaluated the neuroprotective mechanism of lacosamide in MES induced seizures in rats. MES induced seizures lead to increased oxidative stress and activation of neuroinflammatory pathway. Treatment with lacosamide showed a significant reduction in seizure activity, decreased the lipid peroxidation and ameliorated the oxidative stress. The inflammatory processes in the brain contribute to the etiopathogenesis of seizures and epilepsy and this is increasingly recognized as a result of supportive evidence in experimental models and in the clinical setting. In this study, we found a significant increase in inflammatory mediators after MES seizures. However, the administration of lacosamide abolished the activation of neuroinflammatory cytokines. These results indicated the neuroprotective potential of lacosamide by preventing oxidative neuronal damage and activation of brain inflammatory mediators in seizures.

Disclosures: B. Kumar: None. B. Medhi: None.

Poster

497. Anticonvulsant Pharmacological Therapies

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 497.20/H32

Topic: C.07. Epilepsy
**Support:** NINDS Grant 1U54NS079202

**Title:** Diazepam and midazolam effectively terminate tetramethylenedisulfotetramine-induced status epilepticus and enhance survival in mice

**Authors:** *D. ZOLKOWSKA¹, D. A. BRUUN², C. A. BOOSALIS², B. HAMMOCK³, P. J. LEIN², M. A. ROGAWSKI¹;*
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**Abstract:** Tetramethylenedisulfotetramine (TETS) is a highly lethal neurotoxic rodenticide that is believed to act as a noncompetitive antagonist of GABA-A receptors. Severe TETS intoxication has been reported to produce refractory convulsive status epilepticus (SE). Here we characterized the therapeutic efficacy of the benzodiazepines diazepam and midazolam in a mouse model of TETS-induced SE. Mice were implanted with right frontal and parietal cortical screw EEG electrodes. An EMG electrode was implanted in the neck. Recordings were conducted with respect to a screw reference electrode situated in the left parietal cortex. After a recovery period, SE was induced with pretreatment of mice with a single dose of riluzole (10 mg/kg, IP) 10 min prior to administration of a lethal dose of TETS (0.2 mg/kg, IP). Riluzole does not inhibit GABA-A receptor antagonist seizures but does protect against the rapidly lethal effects of TETS in mice, allowing persistent SE. Animals were monitored with a video-EEG system. Seizure activity was scored by visual analysis of the behavioral seizure activity supplemented by EMG and by EEG. Diazepam or midazolam were administered at the human-equivalent doses of 1.8 mg/kg at either 10 or 40 min after the first myoclonic twitch. Diazepam was administered intraperitoneally (IP) whereas midazolam was administered intramuscularly (IM). Latency to cessation of SE is defined as the interval between the first behavioral myoclonic twitch and termination of seizure activity. Vehicle treatment failed to terminate TETS-induced SE, resulting in mortality in more than 80% of the animals within 24 h. Diazepam and midazolam both effectively terminated SE. The latency to cessation of seizure activity was distinctly shorter when the treatment was administered early (10 min) than late (40 min). Either early or late diazepam prevented mortality in 100% of animals. Midazolam prevented mortality in 70% and 80% of animals when administered early and late, respectively. Animals rescued from TETS-induced SE by diazepam or midazolam exhibited reactive astrogliosis and microglial activation in some brain areas as determined by GFAP and Iba-1 immunoreactivity for at least 72 hours. In summary, both midazolam (IM) and diazepam (IP) terminated TETS SE and markedly increased survival. Midazolam has excellent bioavailability when administered IM whereas diazepam is poorly absorbed IM necessitating administration by a different route, in this case IP. Both treatments may be effective in the treatment of TETS-induced SE. Midazolam despite being slightly less effective in promoting survival may be of particular utility because it can easily be administered in the field.

Poster

497. Anticonvulsant Pharmacological Therapies

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 497.21/H33

Topic: C.07. Epilepsy

Support: NIH NS066392

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NSF IOS- 0843585

Title: Ketogenic diet reduces the magnitude, but not maintenance, of hippocampal long-term potentiation in freely behaving juvenile rats

Authors: J. L. KORANDA, *D. N. RUSKIN, J. BLAISE, S. A. MASINO; Trinity Col., Hartford, CT

Abstract: Ketogenic diets are low-carbohydrate, sufficient protein, high-fat diets with anticonvulsant activity and used primarily as a treatment for pediatric epilepsy. The anticonvulsant mechanism is thought to involve elevating inhibition and or otherwise limiting excitability in the brain. Such a mechanism, however, might also significantly affect normal brain activity and limit synaptic plasticity, effects which would be important to consider in the developing brain. To assess ketogenic diet effects on synaptic transmission and plasticity, electrophysiological recordings were performed at the perforant path/dentate gyrus in awake, freely behaving juvenile male rats. Electrodes were implanted one week prior to recording. Animals were fed regular chow or a ketogenic diet ad libitum for three weeks before recording. Although the ketogenic diet did not significantly alter baseline excitability (assessed by input-output curves) or short-term plasticity (using the paired-pulse ratio), it did reduce the magnitude (roughly by half) of long-term potentiation at all poststimulation time points out to the last time measured (48 h). The results suggest an effect of ketogenic diet-feeding on the induction magnitude but not the maintenance of long-term potentiation. The lack of effect of the diet on baseline transmission and the paired-pulse ratio suggests a mechanism that limits excitation preferentially in conditions of strong stimulation, consonant with clinical reports in which the ketogenic diet alleviates seizures without a major impact on normal brain activity.
Limiting plasticity in a seizure-susceptible network may limit seizure-induced epileptogenesis which may subserve the ongoing benefit of the ketogenic diet in epilepsy.

**Disclosures:** J.L. Koranda: None. D.N. Ruskin: None. J. Blaise: None. S.A. Masino: None.

**Poster**

497. Anticonvulsant Pharmacological Therapies

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 497.22/H34

**Topic:** C.07. Epilepsy

**Support:** ERANET-NEURON BrIE

**Title:** Is targeting P2X7 receptor an effective strategy to control seizure induction and recurrence?

**Authors:** *L. LIBRIZZI, F. M. NOÈ, M. DE CURTIS; Fondazione Inst. Neurologico C. Besta, Milan, Italy

**Abstract:** Clinical and experimental evidence support the hypothesis that inflammatory mediators contribute to aberrant neuronal excitability in epilepsy. IL-1b synthesis and release are controlled by the complex NLRP3 inflammasome, activated by the P2X7/pannexin-1 pathway during epileptic seizure-induced massive ATP release. Therefore, a pivotal role of P2X7R has been hypothesized in the aetiology of epilepsy (Dona et al., 2009). We tested if blockade of P2X7R could interfere with seizure-induced brain born inflammation by countering ictal events generation itself. Brief application of bicuculline (50µM, 3min) consistently induced focal ictal discharge in the limbic region, as verified by simultaneous electrophysiological recordings of extracellular activity in medial entorhinal cortex (mERC) and CA1, and promoted the release of IL-1b from brain resident cells (Librizzi et al., 2012). Surprisingly, application of the P2X7R antagonists A-438079 (100µM) or BBG (50µM) 10 min before bicuculline application was notable to counteract bicuculline-induced ictal discharge. On the contrary, an increase of the time spent in seizure was often observed. P2X7Rs activation requires high concentration of ATP. We speculated that bicuculline-induced seizure was ineffective in releasing large amount of ATP. We concluded that P2X7 receptors are not strongly involved in GABA-A receptor mediated seizure events generation. We shifted to the KA acute model of seizure in order to facilitate a wider glutamate-mediated release of ATP by both neurons and astrocytes. Application of KA (6µM, 50ml) induced synchronous oscillation in beta-frequency range, leading to a single ictal episode involving mainly hippocampal formation.
Also in this model, seizure events were associated with IL-1b release exclusively in areas involved in seizure generation (Noè et al, submitted). The 30-min infusion via the resident arterial system of the P2X7 receptor antagonist A438079 (100µM) initiated from 10 min before KA application, resulted into an altered synchronous recruitment of frequencies towards beta-activity and into a prevention of the ictal-activity in the 58% of the experiments. In the remaining 42%, synchronized activity at 15-30 Hz in limbic areas evolving to an ictal event occurred. In conclusion, even though NLRP3 inflammasome system has been demonstrated to be critically involved in aberrant neuronal excitability and seizures, its antagonism, through the upstream inhibition of the P2X7 receptors, could not be a promising strategy to control seizures in focal epilepsies. Further research on P2X7R could provide novel targets to control ictogenesis.


Poster

497. Anticonvulsant Pharmacological Therapies

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 497.23/H35

Topic: C.07. Epilepsy

Support: NIH Grant NS075366

Title: Sleep alterations in the gamma2R43Q mouse model of absence epilepsy, and treatment with ganaxolone

Authors: E. WALLACE1, K. MANGAN2, A. NELSON6, J. PFAMMATTER2, R. MAGANTI3, C. CIRELLI4, *M. V. JONES2;


Abstract: Sleep disruption is a trigger for seizures, and epileptic patients often have sleep disorders (Semin Neurol. 29:419), suggesting a "vicious cycle" of interactions between sleep and epilepsy. Absence epilepsy is especially interesting because, like sleep, it involves a) loss of consciousness without convulsions, and b) reverberations between the thalamus and cortex. The gamma2R43Q knock-in mouse model (RQ) of absence epilepsy expresses a point mutation in the GABA-A receptor that causes absence epilepsy in patients. Like the human patients, RQ mice display behavioral arrests concurrent with generalized spike-wave discharges. We previously showed (SfN Abstr. 2013, 627.01) that a) tonic GABAergic inhibition is abolished in
RQ mice in thalamic and cortical neurons, b) pharmacological block of tonic inhibition in wild-type (RR) animals is sufficient to provoke absence seizures, and c) injection of ganaxolone (GANX, a synthetic analog of allopregnanolone that can rescue the loss of tonic inhibition) in RQ mice is sufficient to reduce their seizure frequency by ~60%. Here we asked whether RQ mice might also have sleep disruptions, and whether GANX might be effective at alleviating such disruptions. RR and RQ mice (n=3 each) were monitored with video and EEG recordings. After a baseline period, the RQ mice were injected with GANX (2 mg/kg i.p.). Sleep stages (Wake, NREM and REM) were scored visually from the EEG by an experienced scorer. EEG delta power (0.5-4 Hz) in NREM sleep was normalized by dividing by the non-delta power (6-100 Hz). RQ mice had (p<0.05, Kruskal-Wallis test) briefer Wake durations than RR, which was not reversed by GANX. RQ mice also had shorter durations of single NREM episodes than RR, but this was reversed by GANX. No groups differed in durations of REM. RQ mice experienced shorter "brief awakenings" (<16 seconds) than RR, and this was not altered by GANX, whereas the number of brief awakenings did not differ between any groups. During normal sleep time (daylight), RQ had higher normalized delta power than RR, and this was partially reversed by GANX. Higher normalized delta power in RQ was unexpected given the differences in NREM above, and may reflect changes in other bands that influenced the normalization. Future work will need to examine differences in the full EEG spectra between RR and RQ, in both sleep and epilepsy. Our preliminary results suggest that sleep alterations accompany absence epilepsy in RQ mice, particularly affecting NREM sleep. Some of these alterations can be reversed by GANX. Selective pharmacological manipulation of tonic inhibition may be a useful avenue for treating both seizures and related sleep disorders in the future.


Poster

497. Anticonvulsant Pharmacological Therapies

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 497.24/H36

Topic: C.07. Epilepsy

Title: Evaluation of synthetic derivatives of medium chain triglyceride (MCT) fatty acids in mice

Authors: *J. A. ARAUJO1, J. CASKENETTE2, A. PATRICK2, W. LAU1, L. BALENCT1, J. S. ANDREWS1, S. ANNEDI1, G. A. HIGGINS1;
Abstract: The medium chain triglyceride (MCT) ketogenic (KG) diet is considered among the most effective therapies for certain refractory epilepsies and has also been proposed to be of value for the treatment of pain and inflammation conditions. The MCT KG diet contains medium chain fatty acids (MCFA) with caprylic acid (CA8) and capric acid (CA10) being primary constituents. Acute treatment with CA8 or CA10 increases blood levels of β-hydroxybutyrate (βHB) - a biomarker of ketosis, but both compounds show very modest effects in mouse seizure models compared with synthetic anti-epileptic drugs such as valproate. Recently Chang et al (JPET [2015] 352:43-52.) described a novel series of synthetic derivatives of MCFA with evidence for improved tolerability and efficacy compared to CA8 and CA10, and possible improvement over the MCT KG diet. The present studies benchmarked a selection of these synthetic derivatives to CA8, CA10 and acetone. All compounds were evaluated across; a standardized series of seizure tests (MES, scPTZ, 6Hz, corneal kindling (CK)), a pain model (spared nerve injury), tests of side-effect (body temperature, rotord, motor activity) and blood βHB level. All studies were conducted in male CD-1 mice. Acetone, CA8 and CA10 showed a varying degree of efficacy in the MES, 6Hz and scPTZ tests and increased blood βHB level, but at doses that affected motor function. For example, acetone had a broad spectrum of anti-seizure activity (ED50: MES, 6Hz, CK = 12-20mmol/kg), yet side effects such as reduced rearing was evident at equivalent doses, i.e 20mmol/kg). Anti-seizure effects of CA8 and CA10 were considered marginal, even at doses that were poorly tolerated. In contrast two synthetic derivatives of CA8, 4-EOA and 4-BCCA, showed robust efficacy in the 6Hz, MES, scPTZ models and reduced side effect liability. For example, 4-EOA was active in each of the seizure tests with efficacy and potency significantly superior to both CA8 and CA10 (ED50 = 1-1.5mmol/kg), and a margin of separation improved from acetone. Consequently, these studies support the use of certain synthetic MCFA derivatives as an advance on the MCT KG diet for the treatment of refractory epilepsy and possibly pain and inflammation disorders.

Poster

497. Anticonvulsant Pharmacological Therapies

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 497.25/H37

Topic: C.07. Epilepsy

Support: CURE (Infantile Spasms Initiative)

- Department of Defense W81XWH-13-1-0180 grant
- NINDS 078333
- Heffer Family Foundation
- Segal Family Foundation
- Abbe Goldstein/Joshua Lurie and Laurie Marsh/ Dan Levitz families

Title: The tolerability and efficacy of an mTOR inhibitor Torin1 on spasms in the multiple-hit rat model of infantile spasms

Authors: T. BRIMA1, W. MOWREY2, *S. L. MOSHE3, A. S. GALANOPOULO4;

Abstract: RATIONALE: Infantile spasms (IS) are age-specific epileptic seizures typically seen in West syndrome, an infantile epileptic encephalopathy with poor outcomes. Better therapies for IS are needed. Early spasm cessation may offer some disease modification in IS patients of unknown etiology whereas structural etiologies render IS more refractory to current treatments: adrenocorticotropic hormone (ACTH) or vigabatrin. The multiple-hit rat model of IS is a chronic model of refractory IS due to structural lesions. In the multiple-hit model, there is overactivation of mTOR pathway. Pulse mTOR inhibition with rapamycin stops spasms and improved learning. To identify mTOR inhibitors with better therapeutic potential for IS, we tested the acute effects
of Torin1, a dual TORC1/TORC2 inhibitor, in the multiple-hit rat model. METHODS: On postnatal day 3 (PN3) Sprague-Dawley male rats received doxorubicin (DOX) (right intracerebroventricular) and lipopolysaccharide (LPS) (right intraparietal) infusions under isoflurane anesthesia, followed by intraperitoneal (i.p.) p-chlorophenylalanine on PN5. Daily monitoring of weights, surface righting time, open field activity, and negative geotaxis were recorded from PN3-PN5. We followed a randomized, blinded, dose and time response study design, administering a single injection of Torin1 (1, 5, or 10 mg/kg i.p.) or its vehicle on PN4, after spasms onset. Intermittent video-monitoring was done on PN4 and PN5 for pre- and post-injection scoring of spasms. Statistics included linear mixed model analysis of raw and normalized log-transformed spasm rates accounting for repeated observations. 11-4 rats were included in each group. RESULTS: All doses of Torin1, given after spasms’ onset, acutely reduced both the raw and normalized frequencies of spasms in the multiple-hit model, for up to 5 hours after administration, dose-dependently and was well tolerated. DISCUSSION: Our results further support the role of mTOR signaling in the pathogenesis and treatment of IS in the multiple-hit model. All doses of Torin1 successfully reduced spasms in the multiple-hit model of refractory IS within the first 5 hours post injection, dose-dependently. Torin1 is a promising candidate drug for further evaluation for the treatment of refractory IS.

Disclosures:  T. Brima: None. W. Mowrey: None. S.L. Moshe: None. A.S. Galanopoulou: None.

Poster

497. Anticonvulsant Pharmacological Therapies

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

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Topic: C.07. Epilepsy

Support: CNPq Grant 304029/2010-6

CNPq Grant 476744/2012-1

FINEP Grant

FAPERGS Grant 101019-1

Title: Paradoxical effects of subchronically and acutely administered cyclooxygenase-2 inhibitors on pentylenetetrazol (PTZ)-induced seizures

Abstract: Cyclooxygenase-2 (COX-2) has been recognized a critical step in prostaglandin synthesis and inflammation. Accordingly, COX-2 inhibitors have been proposed to reduce neuroinflammation and its physiopathologic consequences. However, there is a dispute regarding the role of COX-2 in seizures, because there are reports that COX-2 inhibitors may either decrease or facilitate convulsive episodes. Thus, considering the current discrepancy regarding the pro- and anticonvulsant effect of COX-2 inhibitors, and the use of different drugs and administration regimens, the aim of the current study was to investigate whether acute and subchronic administration of celecoxib, etoricoxib and nimesulide alter PTZ-induced seizures in mice. Adult male Swiss mice were used. In acute experiments, vehicle (0.1% carboxymethylcellulose plus 5% Tween 80, 10 ml/kg, p.o.), celecoxib, etoricoxib or nimesulide (0.2, 2 or 20 mg/kg, p.o.) were administered 60 minutes before PTZ (50 mg/kg, i.p.). In subchronic experiments, mice received vehicle, celecoxib, etoricoxib or nimesulide (0.2, 2 or 20 mg/kg, p.o., daily) for 14 days. On the 15th day the animals were challenged with PTZ (50 mg/kg, i.p.) and monitored for 20 minutes for the appearance of seizures. The latency to myoclonic and generalized tonic-clonic seizures, number of seizure episodes, total time spent seizing and Racine score were recorded. The acute administration of nimesulide dose-dependently decreased PTZ-induced myoclonic jerks [H(3)=11.63; p<0.05], generalized tonic-clonic seizures [H(3)=9.44; p<0.05] and number of seizure episodes [F(3,28)=4.2; p<0.05]. Acute celecoxib and etoricoxib did not alter the parameters analyzed. The subchronic administration of nimesulide and etoricoxib significantly decreased the incidence of PTZ-induced tonic-clonic seizures [H(3)=8.3; p<0.05], [H(3)=10.28; p<0.05]. However, subchronic celecoxib significantly decreased the latency to PTZ-induced tonic-clonic seizures [H(3)=8.73; p<0.05]. Our results suggest that the effect of COX-2 inhibitors on seizures may vary depending on the drug, dose and regimen of drug administration. Such a discrepancy between the effects of COX-2 inhibitors may be due to differential COX-2 selectivity, putative interaction with other targets or pharmacokinetic issues.

Title: Attenuation of corneal kindling progression by 2-deoxy-D-glucose treatment is reflected in 18-F-fluoro-deoxy-D-glucose brain kinetics

Abstract: Rationale: Hitherto, processes leading to acquired epilepsy cannot be prevented. Recent studies suggest that modulation of cerebral glucose metabolism by 2-deoxy-D-glucose (2-DG, an inhibitor of glycolysis) treatment might exert antiepileptogenic effects. In this study, we used 18-F-FDG PET to investigate effects of 2-DG treatment during epileptogenesis.

Methods: 6Hz-corneal kindling as epileptogenesis model was performed in male NMRI mice by twice daily electrical corneal stimulation for 21 days. Saline (n = 12) or 250 mg/kg 2-DG (n = 18) were injected i.p. 18 h before baseline scans and subsequently 1 min after each stimulation. Seizure response was scored using a modified Racine scale. Twelve additional mice received 2-DG without kindling. Dynamic 60-min 18-F-FDG PET/CT scans were acquired at baseline and inter-ictally on days 10 and 17. A standard MRI-based brain atlas was used to quantify 18-F-FDG uptake (%ID/cc). Kinetic modelling (FDG-2-compartment model) using an image-derived input function (vena cava caudalis) was performed to evaluate glucose metabolic rate MRGlu and uptake rate constant Ki. Results: Kindling progression was attenuated in the 2-DG-treated group, mainly in the early phase (up to 29.3 ± 11.8%, p=0.0009). Corneal kindling in combination with 2-DG treatment increased 18-F-FDG uptake by up to 36.0 ± 10.3% (p=0.0016) at day 10 in hippocampus and at day 10 and 17 in amygdala, striatum, cortex and cerebellum, compared to the unkindled 2-DG treated group. At day 10, the 2-DG treated kindling group showed an up to 1.56 fold increase in influx constant Ki compared to the unkindled group as well as a higher MRGlu at day 17 than the saline-treated kindling group. Kindling progression without treatment altered neither 18-F-FDG uptake nor kinetic parameters. Conclusions: Increases in F-18-FDG brain uptake are present only in mice undergoing both, 2-DG-treatment and corneal kindling. Increased F-18-FDG influx is associated with ameliorated kindling progression. As 2-DG treatment might reflect the crucial mechanism of ketogenic diet, an effective treatment scheme for pharmacoresistant epilepsy, ongoing investigations in the intrahippocampal kainate model of epileptogenesis will include a respective treatment.

Title: Dexamethasone and losartan fail to protect blood-brain barrier integrity during early epileptogenesis

Authors: *H. BREUER*1,2, M. MEIER3, W. HÄRTIG2, M. BANKSTAHL2, J. P. BANKSTAHL1;

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Abstract: Objective: Epileptogenesis can be initiated by primary brain insults and results in the occurrence of spontaneous recurrent seizures. Accumulating evidence suggests that insult-associated blood-brain barrier (BBB) leakage is a key step in the initiation of epileptogenesis and may contribute to disease progression. Therefore, BBB-protective treatment might prevent or attenuate epileptogenesis. In this study, a non-invasive theranostic imaging marker for BBB leakage was used to evaluate two potentially BBB-protective treatments, losartan and dexamethasone, during early epileptogenesis. Materials and methods: As primary brain insult, a 90 min status epilepticus (SE) was induced by fractionated pilocarpine injection (i.p.) in 24 adult female Sprague-Dawley rats. Ten rats were treated with dexamethasone (3 and 24 h post SE, 8 mg/kg i.p.; 48 h post SE, 4 mg/kg i.p.) or losartan (30 min post SE, 50 mg/kg i.p.; 24 h till 5 d post SE, 5 mg/kg i.p. b.i.d.; days 6 till 14, 100-150 mg/d via drinking water). Animals were scanned baseline and 48 h after SE, and the losartan-treated group additionally 5 h and 10 d after SE. 7T small animal MRI setup included a T2 sequence, followed by i.v. infusion of gadolinium (Gd)-DTPA and post-contrast T1-MDEFT. For data evaluation, MRI images were analyzed by co-registration with a rat brain atlas and T1- and T2-signal increase (normalized to pons) was calculated. Next, animals received green fluorescent FITC-albumin (i.v.) for subsequently intended histological evaluation of BBB-integrity, followed 2 h later by perfusion with...
paraformaldehyde. Results: In controls, T1-signal after contrast agent and T2-signal increased up to 281% (p <0.0001) and up to 42% (p <0.0001), respectively, in typically SE-affected brain regions like hippocampus, thalamus and entorhinal cortex 48 h after SE. Neither dexamethasone nor losartan treatment influenced brain uptake of Gd-DTPA at 48 h after SE compared to controls. At 5 h and 10 d after SE, no MRI signal increase was present in controls, while T1-signal was increased in various brain regions of losartan-treated rats. Furthermore, in both dexamethasone- and losartan-treated animals, higher T2-signals were present in distinct brain regions at all investigated time points whereas controls did not show significant increases. Histological analysis of serial brain sections is currently performed. Conclusions: Contrast-enhanced MRI reveals BBB leakage 48 h after SE, which could not be counteracted by two potentially BBB-protective treatments. Furthermore, our data suggest that both treatments can exacerbate SE-associated cerebral edema in the pilocarpine post SE rat model.


Poster

498. Human Epilepsy

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 498.01/H41

Topic: C.07. Epilepsy

Title: Seven year retrospective analysis of acute seizure management in children with intravenous levetiracetam

Authors: *B. F. KIRMANI, ESQ1,2, P. LAKIREDDY3, A. SARODE4, O. KHAN5; 1Neurology, T, Georgetown, TX; 2Scott and White Neuroscience Institutetexas A & M Hlth. Sci. Ctr. Col. of Med., Temple, TX; 3Pediatrics, McLane Children's Hosp. and Texas A & M Col. of Med., Temple, TX; 4Baylor Univ., Temple, TX; 5Univ. of Chicago, Chicago, IL

Abstract: Intravenous Levetiracetam was approved in United States in August 2006 for patients aged 16 years and above. We retrospectively analyzed data at our institution of children who received intravenous levetiracetam for acute seizure management since not much information is available. Methods: A retrospective chart review was conducted on all children less than 18 years who received intravenous levetiracetam at Scott and White Hospital/ Texas A & M HSC College of Medicine, Temple, TX. Subject data were acquired from electronic medical records. Approval of this retrospective analysis was given by our hospital’s institutional review board. Results: We retrospectively analyzed 80 patients who met our inclusion criteria for neonatal...
seizures, status epilepticus and acute repetitive seizures and received intravenous levetiracetam from January 2008 to August 2014. The loading dose of intravenous levetiracetam was 50mg/kg in most patients followed by a maintenance dose of 25 mg/kg every 12 hours. The variables analyzed included clinical data, electrographic documentation, indication of initiation of this medicine, adverse events and seizure control at 6 month well child visits. Response to levetiracetam was favorable. 63 out of 80 patients reached seizure freedom within 24 hours and 14 within 48 to 72 hours. Seizures continued in one patient and two patients died because of seizure activity on three anticonvulsants. No serious side-effects were apparent. Patients were discharged on oral levetiracetam and did well at 6 month clinic visit. Conclusions: Intravenous Levetiracetam seems to be efficacious in acute seizure management in children.

Disclosures: B.F. Kirmani: None. P. Lakireddy: None. A. Sarode: None. O. Khan: None.

Poster

498. Human Epilepsy

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 498.02/H42

Topic: C.07. Epilepsy


DARPA Grant: REMIND

Title: Evaluating the seizure suppression effect induced by electrical stimulation: an in vitro study using human hippocampal tissue

Authors: *M.-C. HSIAO¹, P.-N. YU², D. SONG², C. LIU², C. HECK², T. W. BERGER²;
¹Biomed Engin, ²USC, Los Angeles, CA

Abstract: In our previous study, an in vitro seizure model from human hippocampal slices has been established. Using a multi-electrode array system, spatio-temporal inter-ictal activity can be consistently recorded in high-potassium (8 mM), low-magnesium (0.25 mM) artificial cerebrospinal fluid with additional 100 µM 4-aminopyridine (4AP). The inter-ictal spikes can be recorded in different subregions including dentate, CA1 and subiculum. Using this preparation, we found that electrical stimulation at the seizure focus can suppress 4AP induced inter-ictal spikes. In order to evaluate the suppression effect more quantitatively and efficiently, we analyzed and compared different features of the pre- and post-stimulation data including mean
spike rate, amplitude, interval and power. Detailed methods of slice preparation, seizure induction, signal processing, and preliminary results will be presented. This lays a foundation for future studies on finding the optimal electrical stimulation parameter for seizure suppression.


Poster
498. Human Epilepsy

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 498.03/H43

Topic: C.07. Epilepsy

Title: Clinic0-radiological profile of neurocysticercosis patients & outcomes at bpkm cancer hospital

Authors: *Q. H. ANSARI;
BPKM Cancer Hosp., Bharatpur, Nepal

Abstract: Clinic0-radiological Profile of Neurocysticercosis patients & Outcomes at BPKM Cancer Hospital QH Ansari1,3, LN Sing1, A Pandit1,2, BK Thapa2. 1- Department of Radiodiagnosis & Imaging, BPKM Cancer Hospital, Bharatpur, Chitwan, Nepal 2- Department of Neurology, BPKM Cancer Hospital, Bharatpur, Chitwan, Nepal 3- Surya Ganga Institute of Medical Science and Technology, Patna, Bihar, India Abstract Neurocysticercosis (NCC) is a major cause of neurological illness worldwide. It is the most common identifiable cause of partial seizure especially in the children of developing world. There is insufficient information about NCC in Nepal. This study was, therefore, conducted to evaluate the clinical, neuro-radiographic and therapeutic aspects of NCC at BPKM Cancer Hospital. Material and Methods: 100 patients with this Neurocysticercosis were studied prospectively in 12 months in the BPKM Cancer Hospital (BPKMCH), a secondary-level-referral hospital in the central Nepal. The diagnosis of NCC was based primarily on the neuro-imaging (CT scan) findings. Results: The patients were predominantly females (nearly 60%) with age ranging from 5 to 70 years. school-age children constituted 35% of the patients. The three common manifestations were seizures (95%), headache and or vomiting (40%). CT scan demonstrated a single parenchymal ring or nodular enhancing lesion (REL) in 84% of cases with perilesional edema in nearly 85% of cases. A large majority of patients were treated only with the anticonvulsant drugs (ACDs) for 9 months. Follow-up with repeat CT after 9 months showed a complete resolution of NCC in most of the cases without the need for cysticidal treatment. Conclusion: NCC should be
considered first in the differential diagnosis of new-onset seizure among the patients of developing countries, where taeniasis is endemic. Most of the patients with Neurocysticercosis do not need anticysticercal therapy.

Disclosures: Q.H. Ansari: None.

Poster

498. Human Epilepsy

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 498.04/H44

Topic: C.07. Epilepsy

Title: Therapeutic drug monitoring of oxcarbamazepine in mexican epileptic patients

Authors: *N. CASTRO, D. GONZÁLEZ-ESQUIVEL, H. JUNG; Inst. Nacional De Neurologia, Mexico, Mexico

Abstract: PURPOSE: Epilepsy is one of the most common dysfunctions of the nervous system. Some antiepileptic drugs show a relationship between blood concentration and therapeutic effect then can be evaluated through use of therapeutic drug monitoring (TDM). Oxcarbazepine (OXC) is an analogue of carbamazepine, used for the treatment of partial seizure with or without secondary generalization. The mono-hydroxylated derivative (MHD) is the responsible for the anticonvulsant activity and its concentration is relevant in TDM. The purpose of this study was to investigate the pharmacokinetic variability of oxcarbazepine through clinical therapeutic drug monitoring (TDM) in mexican epileptic patients.

METHODS: Data from serum concentration measurements of MHD were utilized. All included samples were drug-fasting in the morning at steady-state (Cmin).

RESULTS: In total, 217 patient samples were included, men 51.2% and 48.8% women, average age 24.4 years (range 1.7 to 76.6 years). The mean serum concentration/daily dose(Cmin/D)-ratio was similar across genders; 68 % of the patients showed concentrations in the range of 10-25 mg/L. It was found a significative correlation between daily dose and plasma concentration (r= 0.436 p<0.001) although significant differences were found in daily doses of OXC between adult and young patients (p< 0.05. U de Mann-Whitney). It was observed a wide pharmacokinetic variability in C/D-ratio). A significative difference was found in the C/D ratio between patients under monotherapy and those under co-administration with enzyme-inducing antiepileptic drugs (0.85 vs 0.62, p<0.05). A significative correlation between CL/F (L/h) and OXC doses was found r=0.472,( p<0.001) CONCLUSION: Oxcarbazepine is not routinely monitored in patients with epilepsy. The pharmacokinetic variability is extensive and it
would be desirable and relevant to monitor plasma MHD levels in order to individualize therapy, considering the influence of age and enzyme-inducing drugs, for estimation of OXC dose.

**Disclosures:** N. Castro: None. D. González-Esquivel: None. H. Jung: None.

**Poster**

498. Human Epilepsy

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 498.05/H45

**Topic:** C.07. Epilepsy

**Support:** International Cooperation program PCI-2014- IMSS

**Title:** Peripherals biomarkers indicators by neuronal damage and neuronal remodeling in patients with refractory epilepsy

**Authors:** *M. FLORES-MENDOZA*¹,², J. GALLARDO², A. VEGA GARCÍA, Jr³, L. LORIGADOS P.⁴, L. MORALES CHACON⁵, S. OROZCO SUAREZ⁶; ¹Unidad de Investigacion en Enfermedades Neurologica, H.de Especialidades CMN , I, Inst. Mexicano Del Seguro Social, Ciudad DE Mexico, Mexico; ²Unidad de Investigacion en Enfermedades Neurologica, H.de Especialidades CMN , I, Inst. Mexicano Del Seguro Social, Mexico DF, Mexico; ³Ciencias de la Salud, Inst. Politecnico Nacional, Mexico, Mexico; ⁴Unidad de Investigacion Medica en Nefrologia, Inst. Mexicano del Seguro Social, Mexico DF, Mexico; ⁵Ciencias Biologicas y de la Salud, Univ. Autonoma Metropolitana Campus Iztapalapa, Mexico DF, Mexico; ⁶Lab. de Neuroinmunologia, ⁷Programa de Epilepsia, Ctr. Internacional de Restauracion Neurologica, Havana, Cuba

**Abstract:** It has been documented that the atrophy of the hippocampus in patients with temporal lobe epilepsy is due to oxidative stress-induced seizure activity, as well as neuronal remodeling by cell loss and target cells. The aim of this study was to identify peripheral biomarkers indicating damage and neuronal remodeling in blood of patients with temporal and extratemporal lobe epilepsy refractory to treatment who were candidates for surgery. Peripheral blood samples were taken before surgery from epilepsy clinic, a protein extraction were subsequently transferred to a nitrocellulose membrane were incubated with several antibodies of oxidative stress was used; Nitrotyrosine (NT), 4-hydroxynonenal (4HNE) malonaldehyde, to damage, S100β and inflammation pathway of prostaglandins (COX-2) and neuronal remodeling (neurotrophin-3), densitometry analysis of the bands was doing with a Analysis software, using the transferrin how control protein. The results showed an increased expression of proteins of...
oxidative stress NT, 4HNE and malonaldehyde in sera of patients with ELT as compared with sera of healthy controls and extratemporal epilepsy patients, as well as presence of neurotrophin-3 in sera of patients with epilepsy but not in healthy controls. Expression in serum markers of stress and neuronal remodeling that are not found in the serum of healthy individuals indicates that these proteins can be used as biomarkers of damage and remodeling in the nervous system, as a noninvasive test. This project was funded by International Cooperation program PCI-2014-IMSS


Poster

498. Human Epilepsy

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 498.06/H46

Topic: C.07. Epilepsy

Support: Capes

CNPq

Title: Parvalbumin expression in the hippocampus of patients with mesial temporal lobe epilepsy and psychiatric comorbidities

Authors: *J. B. DE ROSS, L. KANDRATAVICIUS, M. R. MONTEIRO, R. C. SCANDIUZZI, C. G. CARLOTTI, Jr, J. A. ASSIRATI, Jr, J. E. C. HALLAK, J. P. LEITE, J. A. S. CRIPPA; Univ. of Sao Paulo, Ribeirao Preto, Brazil

Abstract: Objective: Recent studies have demonstrated distinct neuropathological features in patients with mesial temporal lobe epilepsy (MTLE) and psychiatric comorbidities. However, data demonstrating neurotransmitter dysfunctions are scant. Impairment of GABA-mediated inhibition is one of the main hypothesis to explain generation of seizure activity. Interneurons expressing parvalbumin are directly involved in the inhibitory control of pyramidal neurons, and are also involved in the pathophysiology of schizophrenia. The aim of this study was to quantify parvalbumin expression in the hippocampal formation of patients with temporal lobe epilepsy and psychiatric comorbidities. Methods: Retrospectively, 43 cases were selected from patients with pharmacoresistant MTLE who underwent surgery to epilepsy control. Specimens were divided into three groups according to psychiatric diagnosis: interictal psychosis (MTLE + P),
major depression (MTLE + D) and without psychiatric symptoms (MTLE). Tissue from autopsies without epilepsy were used as controls (CTRL). Sections were immunostained with an anti-parvalbumin antibody. Parvalbumin expression was estimated by quantification of positive immunoreactive area, with ImageJ software. Results: Compared to CTRL, MTLE patients showed significant lower parvalbumin expression in CA4, CA3, CA2, CA1 and entorhinal cortex. MTLE + P patients showed reduced parvalbumin expression in hilus, CA4, CA3 CA2, CA1, prosubiculum and entorhinal cortex, compared to CTRL, and in granular layer and hilus, compared to MTLE + D patients, the latter indicating a differential parvalbumin expression between psychiatric comorbidities groups. More specifically, comparing MTLE and MTLE + P groups, a lower parvalbumin expression could be demonstrated in CA1 and subiculum of MTLE + P patients. Interestingly, the subiculum of MTLE groups, unlike the other subfields, presented with the higher parvalbumin expression, even when compared to CTRL group. Conclusions: Our results indicate that patients with MTLE and interictal psychosis have decreased parvalbumin expression in several subfields of the hippocampal formation. This reduction may underlie the association of psychotic symptoms with MTLE, since postmortem studies have demonstrated a reduction in parvalbumin expression in patients with schizophrenia. Finally, the higher expression of parvalbumin in the subiculum of MTLE patients could act as a compensatory mechanism to control hyperexcitability, as the subiculum directly receives inputs from CA1 pyramidal neurons.


Poster

498. Human Epilepsy

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 498.07/H47

Topic: C.07. Epilepsy

Support: CONACYT Grant 84678

FONDOS FEDERALES-INP

Title: Stereological estimates of expression of KCC2 immunoreactive cells in tissue of patients with chronic medically intractable epilepsy
Authors: *L. GRANADOS*¹, T. E. JUÁREZ-ZEPEDA¹, M. RUÍZ-GARCÍA¹, A. MARHXBRACHO¹, R. R. RODRÍGUEZ-JURADO¹, M. ROJAS-MARURI¹, K. JERONIMO-CRUZ¹, L. CARMONA-APARICIO¹, E. COBALLASE-URRUTIA¹, P. DURÁN-HERNÁNDEZ²; ¹Inst. Naciona de Pediatría, Ciudad de México, Mexico; ²Facultad de Ciencias, UNAM, Ciudad de México, Mexico

Abstract: As it is established, NKCC1 and KCC2 cation chloride transporters are membrane proteins which transport chloride within and outside the neurons respectively, regulating thus the intracellular concentration of this ion, which determines the strength and polarity of the gamma-aminobutyric acid (GABA)-mediated neurotransmission. Alterations in homeostasis of these molecules could explain the hyperexcitability observed in epileptic tissue resected in epilepsy surgery. The objective of the present study was to quantify by stereological methods the expression of chloride transporter KCC2 in tissue of epileptogenic zone resected in surgery of pediatric patients with chronic medically intractable epilepsy (n = 6) and tissue obtained from autopsies of infants whose death were not related to a neurological disease (n = 7) (National Institute of Pediatrics and Institute of Forensic Science, México). Tissues were frozen and serial coronal sections (50 µm) were cut and collected. The sections were processed in parallel and incubated with KCC2 antibody, anti-rabbit biotinylated IgG, peroxidase complex and reveal with DAB. A systematic random procedure using optical fractionator (MBF Bioscience) was employed in counting the number of KCC2-immunoreactive (IR) cells in the white matter. Results showed a reduction in the number of KCC2-IR cells present in the white matter of epileptogenic zone of pediatric patients with chronic medically intractable epilepsy. This work was supported by a grant from Consejo Nacional de Ciencia y Tecnología (86784), México and Fondos Federales-INP to L. Granados-Rojas.


Poster 498. Human Epilepsy

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 498.08/H48

Topic: C.07. Epilepsy
Support: PAPIIT IN211913
CONACYT 239594

Title: High expression levels of inflammatory-related molecules and nitric oxide synthase 2 and 3 in surgical resection tissue from frontal lobe epilepsy of patients

Authors: *O. F. MERCADO-GOMEZ*¹, L. CÓRDOVA-DÁVALOS¹, D. GARCÍA-BETANZO¹, L. ROCHA², M. ALONSO-VANEGAS³, R. GUEVARA-GUZMÁN⁴; ¹Natl. Autonomous Univ. of Mexico, Mexico City, Mexico; ²Pharmacobiology, Ctr. of Res. and Advanced Studies, Mexico City, Mexico; ³Neurosurg., Natl. Inst. of Neurol. and Neurosurg. “Manuel Velasco Suárez”, Mexico City, Mexico

Abstract: Introduction: Epilepsy is a neurological disorder characterized by recurrent and spontaneous seizures. Several reports have shown that neuroinflammatory process has a crucial role in epilepsy because of contributes to the etiopathogenesis of seizures. On the other hand, it has recently described that nitrosative stress mediated by overproduction of nitrogen reactive species (NRS) could have an important role in epileptogenesis. Although inflammation and NRS are well described in experimental rat models and brain tissue samples from patients with temporal lobe epilepsy (TLE), little information is known about pathological process in the frontal lobe epilepsy (FLE). Objective: Analyze the gene expression of inflammatory-related molecules (Interleukin 1β, Interleukin 6, tumor necrosis factor α, Toll-like receptor, high mobility group box 1, NFκB) and molecules related with nitrosative stress (NOS2 and NOS3) in frontal lobe brain samples. Methodology: Frontal lobe surgical resection tissue (n=4) were obtained from FLE patients and samples from control subjects (n=4) with no neurological disorders were obtained from autopsies at the NINN Hospital in Mexico city. The RNA was isolated using the TRIzol method following manufacturer’s protocol and their integrity was achieved by electrophoresis. 1 µg of RNA was reversed transcripted using ReverAid First Strand cDNA synthesis kit. For quantitative PCR assays, we performed SYBR green chemistry using 200 ng of cDNA with specific primers for each gene and normalized with GAPDH gene. We used the Pfaffl method for calculating fold changes in gene expression. Results: Our preliminary data show that there was a high expression level of cytokines genes (TNFα=1682.6, IL-6=1483.4, IL-1β=659.9 folds); together with an increased gene expression of TLR4 and their agonist HMGB1 (2.1 and 2.8, folds respectively). Interestingly, downstream effector of inflammatory cascade such as NFκBp65 (RelA) was also found increased (6.5 fold) in FLE brains samples. Moreover, gene expression of NOS 2/3, enzymes implicated in nitrosative stress, also were upregulated. Conclusion: Our results indicate that there is general increase in gene expression of inflammatory-related molecules and enzymes related with nitrosative stress in brains samples of FLE patients. Although, inflammatory process has been described in other epilepsies (i.e. TLE), we showed for the first time that FLE occurs similar results suggesting a common pathological cascades that contributing to the generation of seizures and lately, a progressive neuronal cell death in both kind of epilepsies

**Poster**

**498. Human Epilepsy**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#: 498.09/11**

**Topic:** C.07. Epilepsy

**Support:** Epilepsy Research Foundation (UK)  
King Saud University (KSA)

**Title:** Impact of temporal lobe epilepsy with hippocampal sclerosis upon expression of 5-HT3 receptors: potential novel antiepileptic target

**Authors:** *H. A. ALOMAR*¹ ², M. SHEILABI³, A. PRINCIVALLE³, A. MASSOURA¹, R. CHELVARAJAH⁴, H. PALL¹ ⁵, N. BARNES¹;

¹Sch. of Clin. and Exptl. Med., Univ. of Birmingham, Birmingham, United Kingdom; ²Pharmacol. and Toxicology Department, Col. of Pharm., King Saud Univ., Riyadh, Saudi Arabia; ³Fac. of Hlth. and Wellbeing, Sheffield Hallam Univ., Sheffield, United Kingdom; ⁴Neurosurg. Dept., ⁵Neurol. Dept., Queen Elizabeth Hosp. Birmingham, Birmingham, United Kingdom

**Abstract:** Mesial temporal lobe epilepsy with hippocampal sclerosis (mTLE-HS) is a chronic neuropathological disorder affecting primarily the medial part of the temporal lobe, particularly the hippocampus. This disease is characterised by recurrent focal-onset seizures that can be associated with epigastric aura. Of those suffering from this disease, 30% experience pharmacoresistance to antiepileptic drugs (AEDs), which may necessitate surgical intervention to control their seizures and improve their quality of life. Given the important role of 5-hydroxytryptamine (5-HT) in the hippocampus, we are investigating the expression of 5-HT receptors in resected hippocampi from patients with mTLE-HS relative to post-mortem ‘control’ hippocampi from donors without a diagnosed neuropathological condition. In this report, we describe the impact of mTLE-HS on the expression of ionotropic excitatory 5-HT3 receptors assessed by immunohistochemistry, qPCR and radioligand binding. Immunoreactivity for the 5-HT3A receptor subunit was increased in surviving neurones, particularly in the CA2 field of the hippocampus, of resected tissues from patients with mTLE-HS compared to control tissue. This finding agreed with the qPCR (quantify the change) and radioligand binding results (homogenate...
results; Bmax = 59±8 and 126±10 fmol/mg control and mTLE-HS, respectively), and autoradiography results. In conclusion, our study revealed a significant increase in the expression of the 5-HT3A receptor subunit in the hippocampus of patients with mTLE-HS. The 5-HT3A subunit constitutes the key component the 5-HT3 receptor complex to impart functionality to the receptor. The overexpression of these excitatory receptors is consistent with the enhanced neuronal activity associated with this type of epilepsy and they may represent a novel molecular for antagonists to control seizures in patients with mTLE-HS.


Poster

498. Human Epilepsy

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 498.10/12

Topic: C.07. Epilepsy

Support: NIH Grant U01-MH-098953

Title: Long-term human brain cell primary culture from neurosurgical patients


Abstract: Despite the high demand for and potential utility of human primary brain cell cultures in various scientific interests, the methodology has been regarded as extremely difficult to achieve and maintain long-term cultures. With IRB approval, various resected brain tissues, including cortical and hippocampal tissues from different disease cases such as Communicating Hydrocephalus, Epilepsy, Normal Pressure Hydrocephalus and brain tumor, were used for primary brain cell culture. We developed a new methodology for human brain cell culture that allows maintenance of functional brain cells for over 6 months. Briefly, besides the pathological diagnostics of disease, tissues were dissected, followed by enzyme-triggered cell dissociation and plating onto glass coverslips with serum-free media for further experiments. The cellular phenotypes were confirmed by electrophysiology, ion imaging, immunocytochemistry and single
cell transcriptome analysis. Under the established culturing conditions, neurons developed synaptic connections and form neuronal networks. This new culturing protocol allows us to perform long-term experiments on human brain cells including neuron and non-neuronal brain cells with various pharmacological manipulations. This new protocol will open up the possibility for various applications originating from resected human brain tissue for physiology, pharmacology and genomic studies. Funded by: U01-MH-098953


Poster

498. Human Epilepsy

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 498.11/13

Topic: C.07. Epilepsy

Title: Findings morphological of the cortex on patients with drug-refractory temporal lobe epilepsy: his involvement with the background in childhood

Authors: *J. VILLED, SR1, J. DE JESUS-CARPANTA, Jr2, F. FERNANDEZ-VALVERDE3, M. ALONSO-VANEGAS4;

Abstract: Purpose: To assess the morphological findings of the cortex in patients with refractory epilepsy undergoing to surgery of temporal lobe and history of febrile seizures. Method: A retrospective study was performed: Twenty patients with mesial temporal lobe epilepsy (MTLE), four with tumors, two with cortical dysplasias and the average age is 33 years. By prior surgical protocol standardization and candidate’s temporal lobectomy and amygdalohippocampectomy are systematically reviewed clinical records with data in Excel. Subsequently the morphological changes was analyzed by histological techniques; H-E, Amino Cupric and PAS and immunohistochemistry technique (anti-GFAP and NeuN). Results: After reviewing MTLE patients; We found five cases with a history of febrile seizures and them a marked loss of pyramidal neurons, a dissociation nucleus-somatic, interstitial edema, cell dysmorphic, in PAS
technique there are accumulation of mucopolysaccharides was observed in the cytoplasm, the aggregates was found with Amino Cupric, the GFAP expression was increased and NeuN was decreased in cortex of this patients. Conclusion: From this study it can be inferred that a first febrile seizure of long time may be an important antecedent to do MTLE, and not only this, but you should think of other antecedents of the childhood that may be the cause of protracted seizure in after time, as they are believed to be generated morphological and functional alterations in cortex and hippocampus since they are developing their brains.

Disclosures:  J. Villeda: Other; Young Research. J. De jesus-carpanta: Other; Youn research. F. Fernandez-Valverde: Other; Research in neuromuscular diseases. M. Alonso-vanegas: Other; Neurosurgeon and Research.

Poster

498. Human Epilepsy

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 498.12/14

Topic: C.07. Epilepsy

Title: Mutation in mtDNA investigation in children mitochondrial disorders

Authors: *O. V. GLOBA¹, L. KUZENKOVA², E. KOLESNIKOVA², K. SAVOSTYANOVA³; ¹Neuro., ²Genet., ³Scientific Ctr. of Children's Hlth., Moscow, Russian Federation

Abstract: Aim: Mitochondrial diseases represent a heterogeneous group of multisystem disorders which preferentially affect tissues with high energetic demands and associate with stroke, epilepsy and multiorgan damage. Mitochondrial disorders are difficult to diagnose due to extreme genetic and phenotypic heterogeneities. The aim of the study is to examine the clinical spectrum and genetic investigation in children suspected of having mitochondrial disorders. Methods: We analyzed the clinical, electrophysiological, and radiologic data, mitochondrial functional tests, systemic biochemical measurements and genomic mtDNA sequencing in 33 of children 6 months-17 years of ages showing the sights of mitochondrial dysfunction. Results: In 24 paediatric patients the mutation in mtDNA was revealed. 10 children had symptomatic focal epilepsy with different types of seizures. 4 patients had ischemic stroke. 4 patients had stroke and epilepsy simultaneously. 3 patients had dilated cardiomyopathy only. In children with stroke 3 patients had mutation in ATPsynthase6 gene, one in 16sRNA, two of children had mutation in ND1c910T>C(4216T>C),related to Leber disease and one had mutations ND1 c910T>C and in ND2c448A>G also usually related to Leber disease, but without visual deterioration. In one girl of 8 years of ages the CYTB c47A>G (14793A>G) which may seen in Leigh disease also
revealed. In contrary in 2 children with high-frequency MELAS (mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes syndrome), mutation A3243G, A11084G had no stroke, but epilepsy and mental delay. In 2 patients with infantile spasms the ND1c910T>C(Leber mutation) were found, and the same mutation in one boy with ischemic stroke. Three patients with seizures had Leigh disease. In epileptic patients the most common mutations were in ATP6 334 A>G, ND1,ND2 or ND3 340A>G(rare) usually in combination, one patient with epilepsy and stroke had tRNAlys,COX and 16SRNA mutation. The ATP6 mutation was revealed in most of the children (13 patients) with epilepsy, stroke, epilepsy and stroke and dilated cardiomyopathy. The rest of children presented no mutation but polymorphisms Conclusion: The genotype-phenotype correlation in children with mitochondrial mutation are not direct in many cases. Many mutations are not specific to clinical manifestations. But the recognition of mitochondrial origin and initiation of etiological treatment may improve prognosis.

Disclosures: O.V. Globa: None. L. Kuzenkova: None. E. Kolesnikova: None. K. Savost'yanov: None.

Poster

498. Human Epilepsy

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 498.13/I5

Topic: C.07. Epilepsy

Support: Clinical and Translational Science Institute of Southest Wisconsin

Title: Pharmocogenomics: a new tool to predict efficacy of anti-seizure drug treatment

Authors: *C. J. MARCUCCILLI*1, T. ZEMBLES3, T. SANDER4, P. MONRAD4, D. P. BICK5, S. O'CONNOR6, M. ZUPANC7, A. K. TRYBA2;
1Associate Professor, Dept Pediatrics, 2Dept Pediatrics, The Univ. of Chicago, Chicago, IL;
3Pharmacol., 4Children's Hosp. of Wisconsin, Milwaukee, WI; 5Genet., Med. Col. of Wisconsin, Milwaukee, WI; 6Northwestern Univ., Chicago, IL; 7UC Irvine, Irvine, CA

Abstract: Epilepsy is one of the most prevalent neurological diseases, affecting about 1-3% of the world population. Unfortunately, the mechanisms underlying seizures are not well understood and drug and surgical treatments are unsuccessful in a significant fraction (~30%)
of people living with epilepsy. Current standard of care pharmaceutical methods to treat epilepsy typically entail a “try it and see if it works” approach. Most anti-epileptic drugs (AEDs) are metabolized by cytochrome P450 (CYPs) enzymes and single nucleotide polymorphisms (SNP) in CYP enzymes are thought to alter the efficacy of AEDs in terms of preventing seizures. We used genomic testing to identify SNP variants in CYP enzymes of both epilepsy patients and first time seizure clinic patients. We found that pediatric epilepsy patients that carry the CYP2C19*2 allele have seizures that are extremely sensitive to and more frequently controlled by Phenobarbital (PHB), compared to patients with the reference allele (*1) or other alleles (*4, *17), regardless of PHB plasma concentrations, seizure phenotype or cause of their seizures. Approximately 85% of patients with the CYP2C19*2 allele (n=17 of 20 tested) have seizures controlled by PHB compared to 40% of patients with the reference or other alleles (n=14 of 35 tested). Seizure control was defined as being seizure free for at least 6 months or having a greater than 50% reduction in seizure activity. In a test for significance of difference between the two proportions, these results are highly significant (z=4.53; p<0.0001). In 2 patients with refractory epilepsy, the CYP2C*19 allele was identified prospectively and both patients became seizure free upon initiation of PHB, one for over 2 years. As CYP enzymes control the rate of AED metabolism, SNP variants in CYP genes may cause either slow AED metabolism or rapid AED metabolism. However, the increased efficacy of phenobarbital treatment in patients carrying the CYP2C*19 allele was independent of PHB blood concentrations. PHB is also metabolized by CYP2C9. However, the CYP2C9*2 allele to this gene did not predict efficacy as 6 out of 12 patients with the CYP2C9*2 allele responded to PHB while 24 out of 39 patients with the reference allele or other variants responded to PHB (z = -1.22; p = 0.22). Thus, we are using genomic approaches to test the hypothesis that epilepsy patients with the CYP2C*19 allele also carry a genetic variant of phenobarbital drug targets (e.g. GABA receptors) that can account for the observed increase in seizure control with PHB treatment. To our knowledge, these are the first data to support the use of a genetic test that could predict AED efficacy.


Poster

498. Human Epilepsy

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM
Title: Subcortical shape modeling provides sensitive markers of structural abnormality in non-lesional temporal lobe epilepsy

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Abstract: Mesial temporal lobe epilepsy is the most prevalent form of focal epilepsy in adults. The disorder is frequently characterised by hippocampal sclerosis (HS), but many non-lesional MTLE patients (MTLE-NL) have no visible abnormalities on standard MRI. Diagnosis of MTLE-NL is often associated with poor surgical outcome relative to MTLE with HS. Traditional volumetric analyses have identified marginal structural differences in the hippocampus, thalamus and amygdala in MTLE-NL, but gross volumetric measures may overlook subtle or localised effects. We applied a novel shape analysis method in a group of 40 MTLE-NL patients (male/female: 13/27; mean age=33.44 years) and 70 healthy controls (male/female: 40/30; mean age=34.75 years). Using FreeSurfer v5.3.0, we reconstructed 14 major subcortical structures from each patient’s T1-weighted image (see Fig. 1A). With a validated surface-based parametric mapping algorithm, we mapped the surface dilation factor (Jacobian determinant) that expresses local area expansions and contractions relative to an average surface. The pointwise Jacobian values of all MTLE-NL patients and controls were predicted from a multiple linear regression model including age, sex, handedness and intracranial volume as covariates. All results were corrected for false discovery rate (q=0.05). MTLE-NL patients showed relative surface area contractions in anterior, medial and posterior portions of the left/right hippocampi, surface area expansions in the medial/anterior hippocampi, surface contractions in the posterior/medial sections of the left/right thalami, and small clusters of surface expansion in the posterior-inferior right thalamus (p<0.05, FDR-corrected) (see Fig. 1B-1D). Pharmacoresistant patients also showed surface area contractions in the right accumbens (p<0.05, FDR-corrected). This high-dimensional shape mapping technique detects subtle morphometric differences in MTLE-NL that are overlooked using global comparisons. The method offers a sensitive marker of disease burden in epilepsy and other neurological disorders.
**Disclosures:**  
C. Whelan: None.  
C.R.K. Ching: None.  
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**Poster**  
**498. Human Epilepsy**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 498.15/17

**Topic:** C.07. Epilepsy

**Title:** Hippocampal and cortical expression of heat shock proteins in mesial temporal lobe epilepsy and their relation to seizure outcome

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**Abstract:** Epilepsy is a disorder of the brain characterized by an enduring predisposition to generate epileptic seizures. An epileptic seizure is a transient occurrence of signs and/or symptoms due to abnormal excessive or synchronous neuronal activity in the brain. More than 50 million people worldwide have epilepsy. In most cases, epilepsy can be successfully treated
with anti-epileptic drugs, but many as 20 to 40 percent of patients with epilepsy are likely to have refractory epilepsy. Epilepsy is divided into focal and generalized according to the mode of seizure onset as well as into genetic, structural/metabolic, or unknown according to the underlying cause or etiology. Focal-onset epilepsies account for about 60% of all adult epilepsy cases, and temporal lobe epilepsy (TLE) is the most common type of focal epilepsy referred for epilepsy surgery and often refractory to antiepileptic drugs (AEDs). Heat shock proteins are conserved proteins, some of them are expressed constitutively and some are induced by stressful stimuli such as high temperatures, ischemia and damage. In the nervous system, the HSPs are induced in a variety of pathologic states, including cerebral ischemia, neurodegenerative diseases, epilepsy, and trauma. Expression has been detected in a variety of cell populations within the nervous system, including neurons, glia, and endothelial cells. Several groups using various models (in vivo and in vitro) of experimental nervous system stress and injury have shown neuroprotective properties of HSPs. In human studies there was found an increase in the Hsp70 levels in CSF depending on the severity of seizures; also this increase was found in key hippocampal subfields and reflects epileptogenicity and poorer outcome of epilepsy surgery. Plasma post-seizure Hsp60 levels in patients were higher than before the seizure and those of controls. We evaluated hippocampus and cortex regions from 14 patients with TLE secondary to temporal mesial sclerosis. The mean age at surgery was 34.57 (19-58) years whereas the mean duration of epilepsy was 27.64 (14-44) years. Seizure frequency varied from 1 to 300 per month. The aim of this study was to find a correlation between the expression of these proteins and the surgical outcome evaluated by ILAE scale (1 to 5, being 5 the worst outcome). We used Spearman’s rho and found negative correlation (-.491 P=0.075) in the expression of Hsp60 and a positive correlation (0.161 P=0.583) in the expression of Hsp70, both in hippocampus. The overview of the data shows us a decreased expression of these HSPs in patients with poor outcome (Hsp70 in smaller proportion) but these results are not significant; this may be due to the small sample size.


Poster 498. Human Epilepsy

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program# Poster#: 498.16/I8

Topic: C.07. Epilepsy

Support: SHRF 2920
Title: The first dedicated epilepsy brain bank in Canada

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Abstract: Basic research in the field of epilepsy lags behind neurodegenerative diseases such as Alzheimer’s and Parkinson’s disease. One reason for this is the lack of properly stored human epileptic tissue for research. Although there is some history of brain banks containing tissue from epileptic patients in Europe and the United States, this has not been true in Canada. The objective of this initiative was the creation of the first dedicated epilepsy brain bank in Canada. Brain banks typically house post-mortem tissue, but epilepsy is unique among brain diseases in that tissue resection is a therapeutic option for many patients who are treatment refractory. An epilepsy brain bank can therefore include tissue from patients who have experienced such a resection, with the option to follow the patient postoperatively and collect additional data. The Saskatchewan Epilepsy Brain bank is a storage source for brain tissue from epilepsy surgery resections. The tissue is carefully frozen with isopentane and stored at -80 C. Because the tissue it not fixed in formaldehyde, it is available for further research using advanced molecular and genomic methods. An important aspect of our approach is that all patients who donate tissue to the bank participate in a detailed work up prior to surgery, which includes video-EEG, MRI, neuropsychological testing and psychiatric screening, and also possible PET scans and fMRI depending on the case. Patients are then followed after surgery to measure seizure and behavioral outcomes. This provides the opportunity to use the brain bank tissue for studies of the factors that promote behavioral comorbidities in addition to the neural mechanisms of epilepsy and recurrent seizures. To date, we have stored portions of tissue from 5 patients. All patients were assessed by two epileptologists at the Saskatchewan Epilepsy Program. All of them had drug resistant epilepsy and were candidates for epilepsy surgery. The mean age of the patients at the time of surgery was 37.2 ± 13.8 and two were males. One patient had profound developmental delay and three had psychiatric comorbidities. MRI revealed lesions in three patients. Four patients (80%) had temporal resections and one had a fronto-temporal resection. After six months of follow up all of them were seizure free. We are now using the tissue for a series of basic science experiments using proteomics, immunohistochemistry, and molecular biology. We are also interested in identifying biomarkers of refractory epileptic activity. These biomarkers will be translated into therapeutic targets, which will be tested in animal models to determine efficacy prior to clinical trials.

Title: Epilepsy as a model of brain plasticity

Authors: *E. ROGENHOFER*1,2, E. SANTARNECCHI1,3, S. MULLER1, G. VATTI6, D. MARINO6, F. KHERIF1, R. WIEST7, M. SEECK8, B. DRAGANSKI1;
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Abstract: Background: Temporal lobe epilepsy (TLE) has been commonly associated with progressive atrophy in hippocampus and related networks. There is mounting evidence that epileptic seizures are associated with increase in hippocampal neurogenesis. Given the ambiguity of these observations we aimed to answer the question about the trajectory of epilepsy-related hippocampal changes in epilepsy as function of disease progression. Our objectives are to i) detect patterns of structural plasticity in the hippocampus associated with TLE and ii) disentangle how clinical disease characteristics modulate structural plasticity with specific focus on hippocampal morphology. Methods: 128 TLE patients were characterized dependent on the laterality of the epileptogenic focus, on a drug resistance and on the presence or absence of mesial temporal lobe sclerosis (MTS and MRI-) and compared to 120 healthy volunteers. Statistical VBM analysis in the framework of SPM12 is based on T1-weighted MRI data. Patterns of structural plasticity were evaluated using between-groups comparison and correlation with clinical disease parameters including disease duration, age of onset and seizure frequency as
well as hippocampal volume estimates ipsi- or contralateral to the epileptogenic focus.

Results: MRI-TLE is associated to bilateral increases of hippocampal and amygdala volume estimates in comparison to estimates of healthy controls, principally in early stages of the disease. In contrast, hippocampal volumes with MTS continuously decline during disease progression ipsilateral to the epileptogenic focus. Structural increments are located in the bilateral hippocampal head, but structural degeneration in the ipsilateral hippocampal body and tail. Drug resistance is characterized by a neurodegenerative process involving the ipsilateral hippocampus and bilateral thalamic structures. In MTS TLE, lower ages of onset are associated to a decreased volume in the left hippocampus and thalamus beside the right inferior temporal gyrus whereas in MRI-TLE, the age of onset is not linked to regional specific structural plasticity. High seizure frequencies favor a volumetric loss in the limbic and associated network marked by different patterns dependent on drug resistance and obvious MTS. Conclusion: Our findings of seizure-induced hippocampal plasticity are clearly linked to the clinical phenotype in TLE. Bidirectional patterns including volume increase and decrease are associated to structural imprinting of disease properties and appear to represent rather a stage within a progressive continuum of structural plasticity than separating different disease entities in TLE.


Poster
499. Ischemia: Inflammation

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 499.01/110

Topic: C.08. Ischemia

Support: NSERC Research Grant 1502

Title: Effects of Antalarmin treatment on the neuroinflammatory response to hippocampal injury post transient global cerebral ischemia in male rats

Authors: *P. BARRA DE LA TREMBLAYE, K. L. ROSS, H. PLAMONDON; Behavioural Neurosci., Univ. of Ottawa, Ottawa, ON, Canada

Abstract: The innate immune response to ischemic stroke can exacerbate neuronal injury by further damaging or killing nearby neurons and other cell types, in part through recruitment of cytotoxic immune cells. Previous reports from our laboratory indicate that Antalarmin, a specific
antagonist to corticotropin releasing hormone type 1 receptors (CRHR1) is effective in downregulating post-ischemic elevations in CORT secretion in basal and stress conditions. The current study seeks to determine the relative contribution of CRHR1 receptors in the regulation of the response of two major immune cell-types, astrocytes and microglia, in the hippocampus at a long-term interval post ischemia. It is hypothesized that the CRHR1 antagonist Antalarmin will act as a protective agent by reducing the reactivity of astrocytes and microglia in the hippocampus post ischemia. Wistar rats (N=62) were divided into 5 groups, either ischemic or sham groups, receiving the antagonist or the vehicle, and 1 control group. We used immunohistological analysis to characterize the distribution and density of glial fibrillary acidic protein (GFAP), ionized calcium binding adaptor (IBA1) and tumor necrosis factor α (TNFα) in the CA1, CA3 and Dentate Gyrus (DG) sub-regions 30 days post ischemia. Results from our study indicate that global cerebral ischemia led to a significant lasting increase in astrocytes, microglia and TNFα compared to sham and home-cage controls. The blocking of CRHR1 receptors by Antalarmin led to a partial decrease in IBA1, GFAP and TNFα immunoreactive cells in all subregions of the hippocampus. Antalarmin also conferred significant CA1 neuronal protection positively correlated with attenuation of microglia activation. These findings support moderate neuronal protection by Antalarmin, which are congruent with modulatory effects of CRH on the excitotoxic cascade post ischemia.

Disclosures: P. Barra De La Tremblaye: None. K.L. Ross: None. H. Plamondon: A. Employment/Salary (full or part-time); University of Ottawa. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Natural Sciences and Engineering Research Council of Canada (NSERC).

Poster

499. Ischemia: Inflammation

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 499.02/111

Topic: C.08. Ischemia

Support: Academy of Finland

Sigrid Juselius Foundation

Title: Secondary pathology in rat cortical stroke model

Authors: *J. E. ANTTILA, T. KUAN-YIN, K. MÄTLIK, M. AIRAVAARA;
Univ. of Helsinki, Inst. of Biotech., Helsinki, Finland
Abstract: Aims Microglia are the immune cells of the brain and are rapidly activated in pathological conditions including ischemic stroke. The aims of the study were to characterize the inflammatory changes in the rat cortical stroke model and to study the role of microglial activation in secondary pathology. Methods A unilateral cortical infarction was induced in adult male Sprague Dawley rats by transiently ligating the distal branch of the right middle cerebral artery with 10-0 suture and occluding both common carotid arteries, followed by release of the ligature after 90 minutes. Immunohistochemistry on sagittal paraffin sections was performed on days 2, 7, 14, 28 and 60 post-stroke to analyze the amount of activated microglia/macrophages in the stroked cortex and in the non-ischemic areas striatum and thalamus. Also neuronal markers were used. Results In the ipsilateral cortex the amount of phagocytic microglia/macrophages was most abundant on day 7 post-stroke. Microglial activation in striatum and thalamus was more delayed, beginning from day 7 post-stroke and persisting several weeks after stroke. The majority of activated microglial cells in striatum and thalamus were phagocytic after day 14. Interestingly, phagocytic microglia in thalamus was associated with neuronal loss. Conclusions In this focal stroke model the infarction area is restricted to cortex. However, activated microglia and neuronal loss was detected also in distal non-ischemic brain regions such as striatum and thalamus. This may be caused by the retrograde and anterograde degeneration of thalamic neurons after injury of the thalamocortical and corticothalamic connections. Secondary thalamic atrophy and microglial activation has been described also in patients suffering from middle cerebral artery infarction. Drug targeting of the secondary affected areas could provide a novel approach for stroke treatment to prevent the neuronal loss.


Poster

499. Ischemia: Inflammation

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 499.03/I12

Topic: C.08. Ischemia

Support: Heart and Stroke foundation of Canada CIHR

Title: Pro-inflammatory and anti-inflammatory profile of microglia and macrophages after experimental cerebral ischemia in mice
Authors: *J. G. ZARRUK, S. DAVID; Ctr. for Res. in Neurosci., McGill Univ. Hlth. Ctr., Montreal, QC, Canada

Abstract: Microglia and macrophages from the peripheral circulation contribute importantly to the inflammatory response after ischemic stroke. Recent studies have examined macrophage/microglia polarization in brain ischemia, however these studies have not distinguished between the polarization state of microglia and infiltrating macrophages. We have used the LysM-EGFP knockin mouse in which peripheral macrophages and neutrophils are tagged with EGFP to distinguish them from microglia at the site of cerebral ischemia and studied the expression of M1 and M2 polarization markers after a transient Middle Cerebral Artery Occlusion (tMCAO). The middle cerebral artery was occluded in LysM-EGFP mice and reperfusion was allowed 90 minutes later. Animals were trans-cardially perfused and tissue taken at 24h, 72h and 7 days post-tMCAO for immunofluorescence staining. There were very few infiltrating EGFP+ cells in the penumbra or core of the infarct at 24h. Their numbers increased markedly at 72h after reperfusion and comprise of both infiltrating macrophages and neutrophils; and are distributed evenly within the lesion core. At 7 days the EGFP+ cells which at this time point consist mainly of infiltrating macrophages appear to be aggregated and clustered in the core of the infarct. We also did double immunofluorescence labeling for two well established M1 markers (CD86 and CD16/32) combined with Iba1. Our results show that both CD86 and CD16/32 are mainly expressed in Iba1+/EGFP- (microglia) but not in Iba1+/EGFP+ (macrophages) cells. Both markers were found in the border and penumbra regions of the lesion. An additional group of mice was subjected to permanent MCAO and FACS experiments done to characterize the expression of TNF and arginase-1 at 3 and 7 days post-injury. Our results showed that a higher percentage of resident microglia expressed TNF as compared to peripherally-derived macrophages at 3 and 7 days post-ischemia. On the other hand a higher percentage of macrophages expressed arginase-1 at 3 and 7 days after the ischemic insult. These results show that resident microglia become activated and polarized to a predominantly pro-inflammatory M1 state while infiltrating macrophages, which are mainly localized in the core of the lesion appear to be less pro-inflammatory. Additional work is underway to assess the expression of more markers. The use of the LysM-EGFP mice has allowed us to distinguish the differentiation state of microglia and infiltrating peripheral macrophages at the site of cerebral ischemia. Acknowledgments: JGZ is founded by the Heart and Stroke Foundation of Canada

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Poster

499. Ischemia: Inflammation

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM
Program/#Poster#: 499.04/113

Topic: C.08. Ischemia

Support: NIH Grant NS062097
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Title: Pharmacologically induced hypothermia reduces inflammatory response after ischemic stroke in mice

Authors: *M. WINTER¹, J. H. LEE¹, W. CAO¹, Z. Z. WEI¹, X. GU¹,², L. WEI¹,², S. P. YU¹;
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Abstract: Stroke is a leading cause of human death and disability in the US and across the globe, with over 800,000 strokes occurring each year in the US alone. Despite stroke’s widespread public health impact, there are very few effective clinical treatments available for acute stroke patients. Preclinical and clinical studies have shown that therapeutic hypothermia is highly protective against ischemic brain injury. Using novel neurotensin receptor 1 (NTR1) agonists, we have previously demonstrated the protective effects of pharmacologically induced hypothermia (PIH) against brain damage after ischemic stroke, hemorrhagic stroke, and traumatic brain injury (TBI) in rodent models. To better understand the mechanism of PIH-induced neuroprotection, we investigated the effect of the NTR1 compound HPI-201 on inflammatory responses following ischemic stroke in mice. Stroke damage increased microglial activation in the penumbra, with peak activation at seven days post-focal ischemia. HPI-201 treatment (2 mg/kg, i.p, 15 min after the onset of ischemia) induced body temperature reduction to 32-34°C in approximately 30 minutes. The hypothermic effect was maintained for 6 hours by additional HPI-201 injections (1 mg/kg). This treatment significantly attenuated microglial activation, and decreased the expression of proinflammatory cytokines, tumor necrosis factor-α (TNF-α) and interleukin-1 (IL-1β), 3 days after stroke. Expression of monocyte chemoattractant protein-1 (MCP-1) and macrophage inflammatory protein 1 (MIP-1α), key chemokines in the regulation of monocyte migration and infiltration, were attenuated by the HPI-201 treatment. These findings indicate that pharmacologically induced hypothermia using HPI-201 suppresses the post-stroke neuroinflammatory response, which may contribute to the brain protective effect of PIH therapy.


Poster

499. Ischemia: Inflammation
Title: Protease-Activated anti-inflammatory therapy for ischemic stroke changed distribution and activation of microglia cell

Authors: *S. ZHANG, L. KOJIC, Y. WEN, D. QIANG, F. MORIN, A. O. BEUKERS, K. REN, M. S. CYNADER, W. JIA; Brain Res. Center, UBC, Vancouver, BC, Canada

Abstract: Regulation of inflammation in the acute stages of stroke is crucial for neuroprotection. We used a genetically engineered AAV that expressed an anti-inflammatory chimeric protein to protect the brain following MCAO. The vector was injected into the brain 4 weeks before the insult and the anti-inflammatory fragment of the chimeric protein was subject to cleavage and release by brain proteases that are activated during ischemic stroke. Lasting and robust neuroprotection was observed in the pretreated rats, and the degree of protection was related to the distance from the center of injection. Here we explore the mechanisms of the anti-inflammatory protection. The expression and distribution of the released protein was examined and we found that our construct was expressed in both neuronal and microglial cells but rarely in astrocytes. The microglial population was strongly affected in ischemic stroke. In the absence of pretreatment, their cell size and density increased markedly in the ischemic area. Pretreatment with the vector largely prevented these changes. These results indicate that our strategy involving prophylactic delivery of an anti-inflammatory construct released by stroke-activated proteases affects activation and behavior of microglia in the ischemic area. The changes in microglia may be at least partly responsible for the neuroprotection observed. Detailed studies are ongoing to examine the signaling pathways in the changed microglia cells.

Ischemia: Inflammation

Topic: C.08. Ischemia

Title: TREM2 presence in margins of ischemic brain lesions

Authors: C. SEGOVIA¹, C. ZURHELLEN¹, L. BELAYEV², *R. C. SWITZER III¹;

Abstract: The microglia cell surface receptor, TREM2 (triggering receptor expressed on myeloid cells 2) has been identified as a marker for an ‘anti-inflammatory’ state for microglia. Mutations of TREM2 are associated with polycystic disease states (PLOSL) and recently with a small subset of cases with Alzheimer’s disease. Lack of TREM2 interferes with the phagocytic role of microglia in the removal plaques in a mouse model of AD. In the role of TREM2 in the removal of apoptotic cells, the sequelae of events depart from those of ‘customary’ inflammatory means. TREM2 is expressed on microglia cells surface ~300 times more than on astrocytes. In our study to evaluate available antibodies against markers of different activation states of microglia, an anti-TREM2 antibody (Lifespan Biosci.) was applied to brain sections from rats in which the middle cerebral artery had been occluded for 2 hours and then allowed to survive 7 days. At the margin of the ischemic area, where both astrocytes and microglia were prolific and highly hypertrophied, TREM2 positive staining was found as a fibrous meshwork, appearing more astrocyte-like than microglia. Double staining for GFAP and TREM2 displayed co-localization in the TREM2-positive meshwork. That is, TREM2 positive fibers were also positive for GFAP. No TREM2 staining was observed in unaffected regions. Double staining for TREM2 and Iba1 showed cohabitation, but no Iba1 positive profiles were fibrous or co-stained for TREM2. The location of TREM2 at the ischemic zone margin suggests, rather simplistically, a possible merging of phagocytic function of both astrocytes and microglia. In other studies the presence of TREM2 has already been noted in the margin of ischemia/infarction, but not with a co-staining of astrocyte-positive structures. For those studies the TREM2 antibody was from a different source. Other markers associated with TREM2 activation, reactive astrocytes, and microglia should be sought to elucidate this novel appearance of TREM2 immunoreactivity in this model of stroke.


Poster

499. Ischemia: Inflammation

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM
Title: Delayed administration of fingolimod or minocycline attenuates neuroinflammation in a stroke model

Abstract: Stroke is a major cause of long-term disability and death worldwide. Inflammation following stroke has been shown to contribute negatively to stroke outcome. Thus, anti-inflammatory treatments have been studied as a potential therapeutic target. When given within hours of stroke onset, these approaches have been shown in many laboratory studies to be neuroprotective. That is, stroke size and neurological deficits could be improved. Recently, studies have focused on approaches which could be used in the chronic phases of stroke to improve recovery of neurological function (neurorestorative). Here, we studied whether delayed administration of anti-inflammatory agents might prove neurorestorative without being neuroprotective. We first administered two different anti-inflammatory drugs previously shown to be neuroprotective (minocycline and fingolimod) to rats subjected to distal Middle Cerebral Artery Occlusion (dMCAO). Drugs were intentionally delayed by 1 d in order to avoid affecting infarct size (neuroprotection). Behavior tests were performed for 6 weeks after dMCAO to assess changes in motor function. Infarct size was assessed by histology, while immune markers Iba-1 (microglia), CD3 (lymphocytes) and CD11b (microglia) were assessed by immunohistochemistry 4 days after dMCAO. Infarct sizes were not significantly different between vehicle treated and fingolimod and minocycline treated groups. Immunohistochemistry showed reduction in numbers of immune marker positive cells. Numbers of Iba-1 positive cells were decreased by both treatments (p<0.05), CD11b positive cells were decreased in mice treated with fingolimod (p<0.05). However, there were no differences in numbers of CD3 positive cells between any of the groups exposed to dMCAO. Behavioral analysis across multiple faculties (sensory, motor, memory) did not show significant improvement in the
minocycline and fingolimod groups. In spite of an anti-inflammatory effect, delayed treatment failed to improve neurological outcome.


Poster

499. Ischemia: Inflammation

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 499.08/117

Topic: C.08. Ischemia

Support: SFB TRR 43

Title: Differential roles of microglia and monocytic cells in the ischemic brain

Authors: *K. GERTZ¹, N. RICHTER², R. UHLEMANN¹, F. KLEMPIN², L. STAERCK², S. WOLF², W. UCKERT², H. KETTENMANN², M. ENDRES¹, G. KRONENBERG¹; ¹Charité-Universitätsmedizin Berlin, Berlin, Germany; ²MDC, Berlin, Germany

Abstract: After brain damage including cerebral ischemia, microglia encounter their peripheral cousins, the monocytes. Despite belonging to the same family, emerging evidence indicates that the one may differ from the other not only in their ancestry but also in their functional characteristics. Here, we set out to study similarities and differences between resident microglia and invading macrophages as regards the brain’s longer-term response to ischemia. We used bone-marrow (BM) chimerism and viral vectors to manipulate BM derived cells. CSF-1 receptor-eGFP transgenic mice were used as recipient mice to distinguish resident microglia using green fluorescence. Animals were lethally irradiated and transplanted with red fluorescent BM. After reconstitution BM-chimeric mice were subjected to 30 min middle cerebral artery occlusion (MCAo)/reperfusion. Immunohistological analysis on day 7 after MCAo showed that the contralateral non ischemic hemisphere did not contain any DsRed-labeled cells. Here, resident microglia displayed a typical ramified morphology. By contrast, we found numerous DsRed+ cells and eGFP+ cells within the ipsilateral MCA territory. These cells typically showed an activated morphology with thickening and retraction of branches. Approximately 80% of DsRed+ cells and virtually all eGFP+ cells in the ischemic striatum displayed Iba1 immunoreactivity. Furthermore, we performed patch-clamp recordings of microglia and invading BM-derived cells in the ischemic area. The contralateral side served as control. Interestingly,
intrinsic microglia cells in the stroke area showed an inward current comparable to cultured microglia cells and a very small outward current. The invading red fluorescent cells revealed an additional delayed rectifying outward K+ current after MCAo indicative for a higher activation state. We performed genomic profiling of activated resident microglia and invading macrophages. On day 7 after MCAo, CD11b+ GFP+ microglia and CD11b+ DsRed+ macrophages were extracted from ischemic brain tissue. RNA amplification and microarray analyses were conducted. Statistical analysis yielded 472 transcripts for DsRed>GFP as well as 970 transcripts for GFP>DsRed. Functional grouping analysis using Gene Ontology followed by Fisher’s exact test with Benjamini-Hochberg correction was performed. For DsRed>GFP, relevant gene categories included ‘innate immunity’, ‘cell adhesion’ and ‘T-cell-immunity’. Our data show that the pathogenetic role of brain microglia versus invading mononuclear cells diverges markedly after brain ischemia. Invading macrophages show more activation relative to resident microglia.


Poster

499. Ischemia: Inflammation

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 499.09/118

Topic: C.08. Ischemia

Title: Post-ischemic tracking and quantification of immune cell migration from the small intestine to lymph nodes and meninges


Abstract: Stroke is an acute neurological disease with a strong inflammatory component. Peripheral immune cells infiltrate the ischemic brain and contribute to stroke pathology. Whereas the identity of brain infiltrating peripheral immune cells has been elucidated, their origin and trafficking after stroke has not been investigated. Given that the intestinal immune system is the largest immune cell compartment in the body, we tested the hypothesis that gut-resident immune cells, specifically lymphocytes, migrate to the brain after ischemic injury. In order to study the trafficking of gut-derived immune cells, we used mice expressing the photoconvertible protein
Kikume Green-Red, which stably shifts its emission from green to red upon exposure to violet light. Mice underwent laparotomy and the distal small intestine (6 cm) was exposed to violet light using a laser light source (405 nm; 4.9 mW). CD45+ immune cells were isolated from the small intestine immediately, 7 and 14 days after photoconversion and analyzed by flow cytometry. Immediately after photoconversion, 33.5±4.2% (n=3) of CD45+ cells expressed the red variant of the protein (KikR). The conversion was equal among different immune cell populations (T cells, B cells, myeloid cells). No CD45+/KikR+ cells were observed in adjacent segments of the small intestine not exposed to violet light and in the peripheral blood. The frequency of CD45+/KikR+ cells decreased at 7 and 14 days (10.5±4.0% and 4.0±0.1% KikR+; n=3). To investigate whether intestinal immune cells traffic to the periphery after cerebral ischemia, we analyzed KikR+ cells in mesenteric and cervical lymph nodes and in the meninges 16 hours after transient middle cerebral artery occlusion. KikR+ B cells, TCRβ T cells and γδT cells were found in the mesenteric, cervical lymph nodes and meninges at days 7 and 14 days after intestinal photoconversion. The frequency of KikR+ cells peaked at day 7 in mesenteric lymph nodes (1.1±0.2% of CD45+, 1.5±0.3% B cells, 0.9±0.2% TCRβ T cells and 0.7±0.3% of γδT cells; n=3) and in cervical lymph nodes (0.9±0.1% of CD45+, 1.3±0.1% of B cells, 0.6±0.1% of T cells and 0.7±0.1% of γδT cells; n=3). Remarkably, the frequency of KikR+ cells was highest in the meninges where 1.2±0.2% of CD45+, 2.5±0.5% of B cells, 0.5±0.1% of TCRβ T cells and 2.1±1.3% of γδT cells were KikR+ 7 days after intestinal photoconversion (n=3). We conclude that the Kikume Green-Red mouse is a useful model to monitor trafficking of intestinal immune cells to secondary lymphoid organs and the CNS. Intestinal B cells, conventional T cells and γδT cells migrate to lymph nodes and to the meninges, where they may play a role in CNS pathologies such as ischemic stroke.

Disclosures: D. Brea: None. C. Benakis: None. M. Murphy: None. C. Iadecola: None. J. Anrather: None.
Authors: *M. RABENSTEIN*¹, S. U. VAY¹, J. HUCKLENBROICH¹, A. WILLUWEIT², K.-J. LANGEN², G. R. FINK¹, M. SCHROETER¹, M. A. RUEGER¹;¹Dept. of Neurology, Univ. Hosp. of Cologne, Cologne, Germany; ²Med. Imaging Physics (INM-4), Juelich Res. Ctr., Juelich, Germany

Abstract: Background: Osteopontin (OPN) is a phosphoglycoprotein with important roles in tissue homeostasis, wound healing, immune regulation, and stress responses. It is expressed constitutively in the brain and upregulated during neuroinflammatory responses, e.g. after focal cerebral ischemia. Inflammatory mediators secreted by activated microglia recruit neural stem cells (NSC) towards the ischemic lesion site, thus initiating regeneration. The effects of OPN on both NSC and microglia remain to be elucidated and are, accordingly, subject of this study. We aimed at examining the effects of OPN on fundamental properties of both NSC and microglia. Methods: Primary fetal rat NSC and primary rat microglia were cultured and treated with different concentrations of OPN. Following OPN exposure, both cell types were assessed for survival under (ischemic) stress conditions and for proliferative activity. Further NSC-specific assays included migration and differentiation potential. Microglia were specifically tested for their LPS-induced release of the inflammatory mediators NO, TNF-alpha and IL-6. Results: OPN had positive effects on the survival, proliferation, migration, and neuronal differentiation of NSC. At least in part these effects were mediated via the chemokine receptor CXCR4. In microglia, OPN dose-dependently increased survival under stress conditions, while it did not affect proliferation. Additionally, treatment of microglia with OPN led to a significant reduction of LPS-induced release of NO, TNF-alpha and IL-6. Conclusion: OPN had positive effects on the survival of both NSC as well as microglia under the conditions of (ischemic) stress. At the same time, it promoted neurogenesis from stem cells and reduced the secretion of pro-inflammatory mediators from microglia. Data suggest that OPN regulates both NSC and microglia function to support regeneration after cerebral ischemia.


Poster 499. Ischemia: Inflammation

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 499.11/120

Topic: C.08. Ischemia

Support: CCNA/ASRP award
Title: Age-dependent cytoplasmic TDP-43 accumulation in cerebral ischemia

Authors: *S. THAMMISETTY*¹, J. KRIZ², F. CALON³;
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Abstract: Introduction: The biological role of TDP43 in the brain is not well understood and it has been detected recently in pathological inclusions in the cytoplasm and nucleus of both neurons and glia of ALS and fronto-temporal dementia as well as in Alzheimer’s disease suggesting a role in inflammation induced neurodegeneration. Ischemic stroke is the third leading cause of death in western industrialized countries. Experimentally and clinically, stroke is followed by acute and prolonged inflammatory responses. Our initial studies revealed that as in chronic neurodegeneration, ischemic injury is associated with a long term and age-dependent cytoplasmic accumulation of TDP-43. Because stroke represents a major risk factor for development of Alzheimer’s style dementia later in the life we propose that TDP-43 may play a role. Methods: We used WT mice as well as transgenic TDP43A315T mice. The stroke was induced by middle cerebral artery occlusion followed by different reperfusion time periods. *In vivo* bioluminescence imaging: The images are taken using IVIS 200 imaging system and 20 min prior to the imaging session mice will administer with luciferine intraperitoneally (150mg/kg body weight). Immunohistochemistry: paraformaldehyde fixed sections are washed with PBS (1X) for 3time, blocked for 1 hr at room temperature and Primary antibody incubation is carried out overnight, followed by an exact Alexa Fluor 488 or 594 secondary antibodies for 2hr at room temperature. Finally sections are observed under microscope. Protein analysis of the brain lysates are carried out by western blot. Results: Immunohistochemistry data and western blot analysis revealed an age-dependent shift of TDP-43 protein into the cytoplasm after stroke in neurons as well as in glial cells. The aging was also associated with an increase in cytoplasmic TDP-35 fragment involved in the formation of aggregates. *In vivo* imaging data showed there is an up-regulation of TLR2 response in TLR2-TDP43A315T double transgenic mice. Conclusion: Our results revealed a long term and age -dependent accumulation of the TDP-43/TDP-35 protein in the cytoplasm of neurons and some glial cells. Further analysis using transgenic models revealed that overexpression of the human TDP-43 leads to an increase in post-ischemic inflammatory and significant increase in ischemic lesions. Taken together our results suggest a role of TDP-43 in modulation of age-dependent neuroinflammation.

Disclosures: S. Thammisetty: None. J. Kriz: None. F. Calon: None.

Poster

499. Ischemia: Inflammation

Location: Hall A
Title: Interleukin-4, which promotes alternative activation of microglia, increases neutrophil infiltration and exacerbates neuron damage if injected into the brain at the onset of ischemia

Authors: *S. Lively1, S. Hutchings2, L. C. Schlichter1;
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Abstract: After stroke, the CNS undergoes a prolonged inflammatory response that involves primarily three innate immune cell types: resident microglia and blood-derived macrophages and neutrophils. In principle, microglia and macrophages can undergo complex activation processes and display a range of functions from cytotoxic to reparative. It is generally thought that an initial pro-inflammatory response is followed by alternative activation processes that reduce inflammation and promote repair. Experimentally, alternative activation is elicited by the cytokine, interleukin-4 (IL-4). Here, we tested the hypothesis that alternative activation will be beneficial at early times after transient focal ischemia. We stereotaxically injected the vasoconstrictor, endothelin-1, into the rat striatum to induce transient focal ischemia, with or without co-injecting IL-4, and then quantified several aspects of inflammation and damage to neurons and white matter in the ischemic infarct at 1, 3 and 7 days. In the damaged ipsilateral striatum, IL-4 increased transcript levels of several genes associated with alternative activation: ARG1, CD163, CCL22, IL-4rα, STAT 6. However, IL-4 treatment did not reduce the extent of white matter injury, which was indicated by reduced myelin basic protein (MBP) staining and increased degraded-MBP staining. Nor did IL-4 reduce the density of activated microglia/macrophages (based on Iba1 staining and morphology) that infiltrated the ischemic core or the proportion that were phagocytic (ED1-labeled). Surprisingly, at 1 day after ischemia, the infarct of IL-4 treated rats had increased numbers of ED1-positive neutrophils (stained for polymorphonuclear leukocyte antigen), which spatially and temporally corresponded with increased neurodegeneration (FluoroJade B labeling). Because VEGF expression increased after IL-4 treatment, we examined blood vessel architecture and BBB breakdown: no differences were apparent in the treated rats. Together, our results suggest that skewing the brain from a pro-inflammatory classical-activation state to an anti-inflammatory alternative-activation state at the onset of ischemia might not be beneficial.

Disclosures: S. Lively: None. S. Hutchings: None. L.C. Schlichter: None.
Title: Gestational inflammation and neonatal arterial ischemic stroke, a causal connection?

Authors: *C. GUIRAUT¹, N. CAUCHON¹, M. LEPAGE¹, G. SEBIRE¹²;
¹Univ. De Sherbrooke, Sherbrooke, QC, Canada; ²Mc Gill Univ., Montreal, QC, Canada

Abstract: Neonatal arterial ischemic stroke (AIS) affects about 1 out of 3000 newborn per year. It leads to severe complications such as hemiplegic cerebral palsy and/or cognitive impairments. The large cerebral arteries from the anterior system, namely the intra-cranial carotid bifurcation, are the most affected, the ischemic stroke being located in its territory in 85% of cases. The classic pathophysiological hypothesis postulated that the arterial occlusion is caused by emboli from placental origin. However, this pathophysiological mechanism is still unsettled. A new pathophysiological perspective emerged from the epidemiological association between gestational inflammation and neonatal stroke (e.g. prolonged rupture of membrane or chorioamnionitis). We hypothesize that materno-foetal inflammation, induced by gestational exposure to pathogens, leads to a site-specific vasculitis affecting the carotid bifurcation and then triggers a focal thrombosis. Material and methods: Dams were injected with saline or lipopolysaccharide (LPS) from Escherichia coli (200 µg/kg/12h) between gestational day (G) 21 and 22. Brains were harvested at G21, G22 and postnatal day 1 (P1). At P1, a prothrombotic stress (transcutaneous photothrombosis) was applied on middle cerebral arteries to compare its susceptibility to thrombosis between LPS-exposed or unexposed pups. Immunohistochemistry and ELISA detected maternal, placental and fetal/neonatal inflammatory markers. Results: Our results showed a maternal, placental and fetal inflammation mediated by IL-1beta, TNF-alpha and MCP-1. These inflammatory mediators were detected in maternal blood, placentas, fetal blood and fetal cerebral arteries. The arterial wall inflammation correlated with the distribution of human neonatal AIS: intra- vs extra-cranial and anterior vs posterior cerebral arteries showed distinct inflammatory phenotypes. Pups exposed to LPS+photothrombosis are more susceptible to AIS and subsequent motor impairments than those exposed to saline+photothrombosis.
Conclusion: Preliminary results from our new pre-clinical model support our hypothesis of increased susceptibility of anterior cerebral arteries to gestational inflammation, and open a new vasculitic pathophysiological avenue for neonatal stroke.


Poster

499. Ischemia: Inflammation

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 499.14/I23

Topic: C.08. Ischemia

Support: KIOM-2010-2

K12220

K13220

K15310

Title: Salvia miltiorrhiza protects white matter and the hippocampus from damage induced by chronic cerebral hypoperfusion

Authors: *M.-S. KIM1,2, J. BANG3, J. LEE1, H. KIM4, J.-S. HAN2, W. JEON1;

Abstract: Ethnopharmacological relevance: Salvia miltiorrhiza (SM), an herbal plant, is traditionally used in the treatment of cardiovascular and cerebrovascular diseases in Asian countries. SM has multiple biological effects including anti-inflammatory activity. However, no studies have been reported about the anti-inflammatory effects of SM on chronic cerebral hypoperfusion in white matter and the hippocampus. Aim of the study: The present study is aimed at investigating the effects of SM in rats with chronic cerebral hypoperfusion. Materials and methods: Chronic cerebral hypoperfusion was induced in male Wistar rats by permanent bilateral common carotid artery occlusion (BCC Ao). The rats were divided into 3 groups: sham-control, BCC Ao treated with vehicle, and BCC Ao treated with SM. Vehicle or SM (200 mg/kg) were administered daily by oral gavage beginning on day 21 after BCC Ao and continuing to day
Immunohistochemical analyses were used to measure Iba-1-positive microglia and myelin basic protein (MBP) in white matter and hippocampal tissue. In addition, the expression levels of proinflammatory cytokines, including TNF-α, IL-1β and IL-6, and the toll-like receptor (TLR) pathway in the hippocampus, were analyzed by western blot. Results: Administration of SM attenuated the activation of microglial cells in the white matter and hippocampus after BCCAo. SM also prevented neuroinflammation after BCCAo by reducing hippocampal levels of TNF-α, IL-1β and IL-6, and increasing the reduced levels of MBP in the white matter and hippocampus. Further, the administration of SM alleviated the up-regulation of hippocampal TLR4 and myeloid differentiation primary response gene 88 (MyD88) in rats with chronic BCCAo. Conclusions: Our findings suggest that SM may be a promising therapeutic candidate in vascular dementia because of its protective effects against damage to the white matter and hippocampus after BCCAo. Keywords: Salvia miltiorrhiza, Chronic cerebral hypoperfusion, Permanent bilateral common carotid artery occlusion, Myelin basic protein, Toll-like receptor

Disclosures: M. Kim: None. J. Bang: None. J. Lee: None. H. Kim: None. J. Han: None. W. Jeon: None.

Poster

499. Ischemia: Inflammation

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 499.15/I24

Topic: C.08. Ischemia

Title: Targeting ischemic brain injury with cocktail drugs during reperfusion ameliorates delayed neuronal cell death following transient global cerebral ischemia

Authors: *I.-C. I. YU1, J.-H. YEN1, P.-C. KUO1, B. C. HONG-GOKA2, R. D. SWEAZEY1, F.-L. CHANG1;
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Abstract: Out-of-hospital cardiac arrest (CA) affects approximately 330,000 people annually in the United States. With advanced cardiac interventions, ischemic brain injury has become the major cause of death and disability for victims of CA. CA disrupts global cerebral circulation, which results in damage to neurons. Many CA survivors who remain in comatose states after successful cardiopulmonary resuscitation, suffer severe damage of the forebrain while having relatively preserved brainstem function. Even conscious survivors may experience deficits in memory, attention, or executive function after discharge. These poor neurological outcomes
indicate an urgent need for targeted therapies. Post-CA brain injury is heterogeneous and multifaceted. We hypothesized that simultaneously targeting multiple injury cascades during the acute phase can suppress early deleterious events and alleviate ischemia/reperfusion (I/R) damage to neurons. We induced transient forebrain ischemia in mice and intravenously administered a cocktail of three drugs to target multiple injury cascades during reperfusion. Our results showed that cocktail drug administered during reperfusion suppressed early neuroinflammatory events as early as 6 hours after I/R. The production of pro-inflammatory cytokine, TNFα, was suppressed in the cortex and striatum of cocktail drug-treated mice. The expressions of chemokines, Ccl2, Ccl3, and Cxcl2, which attract peripheral leukocyte infiltration into the brain, were significantly suppressed. Reduced production of brain chemokines was found to decrease the infiltration of CD45high/CD11b+ leukocytes, including macrophages and neutrophils, at later time points. At the cellular level, we found the reduction of CD86 activation marker in microglia cells isolated from cocktail drug-treated mice 6 hours after I/R. The cocktail drug suppressed activation of primary cultured microglia in response to LPS in vitro, as shown decreased induction of TNFα and IL-6. Besides inhibiting different steps in neuroinflammation, early cocktail drug treatment was found to reduce induction of oxidative stress sensor, Sestrin 2, in response to excitotoxicity after I/R. Collectively, we found that suppression of multiple deleterious events during the acute phase alleviated damage to hippocampal neurons at 3 days after I/R. In conclusion, the cocktail drug was able to suppress multiple neuroinflammatory and oxidative insults, and to alleviate neuronal cell death when administered early. Our results demonstrate that early targeting of multiple I/R injury cascades during reperfusion has the potential to improve neurological outcomes for victims of CA.


Poster

499. Ischemia: Inflammation

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 499.16/I25

Topic: C.08. Ischemia

Title: Blood-borne monocytes amplify inflammation and transform into microglia after LPS sensitized hypoxic-ischemic brain injury in rodent neonates
**Authors:** Y.-Y. SUN1, J. LEE2, C. BRANDON3, N. ANTHONY2, C.-Y. KUAN1;
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**Abstract:** The brain resident microglia have a mesodermal ontogenic origin, but the relationship between microglia and blood-borne monocytes in the neonatal period, with and without injury, remain contentious. Here we use transgenic monocyte- and microglia-reporter mice and adoptive transfer of genetically marked monocytes to study this issue. We hypothesize that (1) monocytes convert to the brain microglial pool prenatally, but stop doing so soon after birth in normal conditions; (2) neonatal infection-sensitized hypoxic-ischemic (HI) injury resurrects the influx of monocytes; (3) these invading monocytes amplify inflammatory responses and transform into the brain resident microglia. We report five sets of results. First, in E17 bitransgenic CCR2-RFP; CX3CR1-GFP embryos, monocytes (RFP+), microglia (GFP+), and RFP/GFP-double-positive cells were readily detected between the choroid plexus and subcortical white matter. Time-lapse imaging in brain slices indicated the monocyte-to-microglia conversion at this stage. Second, in postnatal P6 brains, EdU+ amoeboid microglial cells (AMC) at the classic “fountains of microglia” sites comprised exclusively CX3CR1-GFP+, but not CCR2-RFP-negative cells, suggesting greatly diminished monocyte influx after birth. Third, in P10 bitransgenic mice, a large number of CCR2-RFP+ monocytes and CCR2-RFP+/CX3CR1-GFP+ hybrid cells were found in the ipsilateral hemisphere of LPS (lipopolysaccharide)/HI-injured brains. The monocytes and monocyte-derived cells express pro-inflammatory cytokines (e.g. IL-1β and TNFα). Fourth, deletion or pharmacological inhibition of CCR2 (with RS102895) greatly diminished the influx of monocytes and inflammatory responses to LPS/HI brain injury, leading to greater preservation of the neural tissue. Finally, when actin-GFP+/CCR2-RFP+ monocytes were intravenously transferred to LPS/HI-injured mice, the expression of CCR2-RFP was quickly down-regulated, while GFP+ monocytes transformed into AMC-like cells at 3 day, and microglia-like cells at 7 day recovery. Together, these results suggest that the blood-borne monocytes contribute to inflammatory responses to neonatal infection/HI injury, and likely transform into microglia, whose impacts on neural development are yet to be determined.

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**Poster**

499. Ischemia: Inflammation

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

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Topic: C.08. Ischemia

Support: NIH R01GM114851

Title: Systemic immune responses to cardiac arrest in mice

Authors: N. BRANDON1, H. DOU2, *Y. XU1;

Abstract: Systemic immune responses to cardiac arrest and resuscitation have both neuroprotective and neurodegenerative effects. Here we examined the time-dependent immune processes in a mouse model of cardiac arrest and resuscitation to map the immune signals and to understand possible pathways for future therapeutic interventions. We hypothesize that cardiac arrest activates specific components of the immune system in a time dependent manner with measurable quantities, which are correlated with the degree of neuronal damage and consequently with functional and behavioral outcomes. Six-minute cardiac arrest was induced in adult male Balb/c mice (Jackson Labs) by an IV injection of a short acting β-blocker (esmolol) along with asphyxia and reversed by a retrograde arterial infusion of oxygenated blood containing a resuscitation mixture of epinephrine, sodium bicarbonate, and heparin. Sham-operated mice had one of their femoral arteries and veins tied off but did not receive drugs other than anesthesia and analgesia. Surgically naïve mice were also used as controls. Bone marrow, blood, spleen, liver and brain were collected on post-surgical days 1, 3, and 5 for flow cytometry and/or histology analyses. In spleen, CD11b+ cells were continuously increased on days 1, 3, and 5 after resuscitation. The ratio of CD4+ to CD3+ T-cells was decreased from 68% of control to 61.25% and 60.25% on days 3 and 5 after resuscitation, respectively. The sham operation slightly increased the ratio of CD4+ to CD3+ T-cells to 71%. In bone marrow, cardiac arrest mice exhibited decreases in CD11c+ cells and increases in CD11b+ cells as compared to the naïve and sham controls. The CD4+ and CD3+ T-cells were clearly increased on post-arrest day 3 but returned to the control levels on post-arrest day 5. In both liver and spleen, cardiac arrest resulted in a depletion of CD3+FoxP3+ and CD8+FoxP3+ T-cells, and a significant increase in CD11b+/Ly6C+ inflammatory monocytes/macrophages and CD11b+/Ly6G+ immune suppressor cells on post-arrest days 3 and 5 as compared to the controls. In addition, the cardiac arrest mice showed decreased CD11b+Ly6C+F4/80+ tissue macrophages in the liver and spleen on post-arrest days 3 and 5. We conclude that specific immune responses to cardiac arrest and resuscitation can be identified, allowing us to design new therapeutic strategies to target immune system as needed. This work was supported by a grant from the NIH (R01GM114851).

Disclosures: N. Brandon: None. H. Dou: None. Y. Xu: None.

Poster
**499. Ischemia: Inflammation**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

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**Topic:** C.08. Ischemia

**Support:** P3SMP3 148367 from the Swiss National Science Foundation and the Swiss Foundation for Grants in Biology and Medicine

**Title:** Modification of commensal gut bacteria induces protection from ischemic brain injury


**Abstract:** Commensal gut bacteria have a profound impact on the host physiology, metabolism and immune function and influence disease processes affecting a wide variety of organs, ranging from the gut to the brain. Given that intestinal microbiota can impact physiology and pathology of the CNS, we sought to determine whether modification of the intestinal flora affects the outcome of ischemic brain injury in a mouse model of transient middle cerebral artery occlusion (MCAo). To modify the gut microbiota composition, C57Bl/6 male mice received a cocktail of antibiotics in the drinking water that target a broad spectrum of bacteria phyla (ampicillin, metronidazole, neomycin, and vancomycin; AMNV). Four weeks later, fecal bacterial DNA was analyzed by quantitative PCR of the 16S ribosomal RNA gene. To rule out off target effects of antibiotics, their administration was discontinued three days prior to MCAo. Infarct volume was measured 3 days after ischemia in cresyl violet stained brain sections. AMNV treatment resulted in a marked reduction of fecal bacterial DNA (6x10^8 vs 2x10^4 copies/mg fecal mass; n=5-10; p<0.001) and a significant decrease of the infarct volume (36±19 mm^3, n=12, mean±SD) compared to control mice (54±21 mm^3, p<0.05, n=12). Recolonization of AMNV-treated mice with wild type flora for two weeks reestablished the number of fecal bacterial copies to control levels (1x10^9 copies/mg; n=4) and abolished the observed neuroprotection (infarct volume: 72±18 mm^3, p<0.05 from controls; n=8). Interestingly, the protective effect was not due to additive effects of different antibiotics, because treatment with V or A alone reduced infarct volume as much as AMNV treatment (controls: 51±24 mm^3, V: 37±23 mm^3, A: 20±7 mm^3; n=15-23/group). The profound neuroprotective effects of intestinal dysbiosis could not be attributed to differences in the levels of cerebral ischemia because cerebral blood flow reduction produced by MCAo was comparable in AMNV-treated mice and controls (controls: 94±2 %, AMNV: 92±2 %, p>0.05; n=12/group). Similarly, AMNV-treatment did not reduce the brain lesions produced by neocortical injection of NMDA (20nmol in 140nl) (controls: 3±1 mm^3,
AMNV: 4±1 mm³, p>0.05; n=5-7/group), suggesting that the reduction of ischemic injury is not mediated by inhibition of excitotoxicity. The composition of intestinal microbiota has a substantial impact on stroke outcome. The mechanisms of the protective effect remain to be defined, but they are unlikely to include vascular effects or protection from glutamate excitotoxicity. Efforts to modify the intestinal flora may provide new preventive approaches to reduce ischemic brain injury in high-risk patients.


Poster

499. Ischemia: Inflammation

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#/Poster#: 499.19/128

Topic: C.08. Ischemia

Support: NIH grant NS073779

Title: Dysregulated cytokine released by activated microglia in the hippocampus of diabetes associated recurrent hypoglycemic rat brain exacerbate ischemic damage

Authors: *V. SHUKLA, A. K. REHNI, K. R. DAVE;
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Abstract: The Dysregulated innate immune response due to microglial activation in the brain exhibit deleterious effects. Earlier published study from our laboratory demonstrated that recurrent hypoglycemia (RH) in a rat model of insulin-dependent diabetes exacerbates cerebral ischemic damage (Stroke, 42, 2011, 1404). We therefore aimed to determine potential involvement of inflammatory mediators released by activated microglia in cerebral ischemic damage in diabetic rats exposed to RH. Naïve and streptozotocin (Stz) diabetic rats were used for the study. To correct hyperglycemia, a subcutaneous insulin pellet was employed about 2 weeks after injection of Stz. Three experimental groups were examined: (1) naïve (non-diabetics, n = 5), (2) insulin-treated diabetics (ITD, n = 3), and (3) ITD+RH (diabetics on insulin therapy experiencing RH, n = 5). RH was induced once a day for 5 consecutive days. Global cerebral ischemia (8 min, 2VO + hypotension) was induced the day after the last hypoglycemia treatment. Animals were euthanized at 24 h post-reperfusion, brains were excised aseptically, and hippocampus were separated. Hippocampal homogenates were centrifuged at 20,000 × g for 15 minutes. The supernatants were used for the study. Western blot was performed using anti-IL-10,
anti-TNF-α and anti-Iba-1 antibodies. β-actin was used as a loading control. Statistical evaluation of the data was performed using ANOVA followed by Tukey’s post hoc test. We observed marked decrease in IL-10 protein level in hippocampus of ITD+RH group compared to ITD (33%) and naïve group (37%). This decline was significant (p < 0.05) relative to the naïve group. A significant marked increase (70%) in the level of TNF-α was observed in the hippocampus of ITD+RH group compared to the naïve group. The levels of TNF-α were higher (19%) in ITD+RH group compared with ITD group. However, the difference was not statistically significant. Compared to the naïve group a significant (p<0.05) increase in Iba-1 level was observed in the hippocampus of ITD+RH (37%) as well as ITD (42%) group. We are currently increasing number of observations in each specific groups to further confirm our results. The enhanced expression of TNF-α in ITD+RH group might be responsible for exaggerating the ischemic damage in this group. Understanding the mechanism by which recurrent hypoglycemia exposure is involved in post-ischemic dysregulation of cytokines (increased TNF-α production and decreased IL-10 response) may help lower the ischemic damage in diabetes.


Poster

499. Ischemia: Inflammation

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 499.20/129

Topic: C.08. Ischemia

Support: AHA fellowship POST20130024

Title: Recombinant tissue plasminogen activator promotes, and progesterone attenuates, microglia/macrophage M1 polarization and recruitment of microglia after MCAO stroke in rats

Authors: S. WON¹, J. LEE², D. STEIN³, *L. WEI¹;
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Abstract: Background: Tissue plasminogen activator (tPA) is one of the few approved treatments for stroke, but its effects on the phenotype of microglia/macrophages are poorly understood. One of its side effects is an increase in the inflammatory response leading to neuronal cell damage and death in the ischemic cascade after stroke. Injury-induced activated microglia/macrophages can have dual functions as pro-inflammatory (M1) and anti-
inflammatory (M2) factors in brain injury and repair. Recent studies show that progesterone (PROG) is a potent anti-inflammatory agent which affects microglia/macrophage expression after brain injury. Purpose: We examined the interaction of tPA-induced expression of microglia/macrophage phenotypes and PROG’s anti-inflammatory effects. Results: tPA treatment increased the recruitment of microglia/macrophages, the polarity of M1 reactions, the expression of MIP-1α in neurons and capillaries, and the expression of MMP-3 compared to vehicle, and PROG modulated these effects. Conclusions: PROG treatment attenuates tPA-induced inflammatory alterations in brain capillaries and microglia/macrophages both \textit{in vivo} and \textit{in vitro} and thus may be a useful adjunct therapy when tPA is given for stroke.

Disclosures: S. won: None. J. Lee: None. D. Stein: None. L. Wei: None.
of the current study was to assess for differences in the effect of microglial suppression on HI-induced brain injury in P9 and P30 mice. We hypothesized that administration of minocycline after HI would result in suppression of microglial activation in both age groups, and would improve brain injury after HI in younger mice. **Methods:** HI was induced in P9 and P30 mice by unilateral carotid artery ligation and exposure to 10% O2 for 50 minutes. Minocycline or vehicle was administered at 2 hours and 24 hours post-HI. Microglia responses and neuronal injury were characterized using flow cytometry and immunostaining at 2 days and 9 days post-HI. T2-weighted MRI was performed at 9 days and 60 days post-HI to assess for HI-induced cerebral volume loss. HI-induced impairments in memory/learning were assessed using Morris Water Maze testing at 2 months post-HI. **Results:** Minocycline administration effectively suppressed the microglial response in P9 and P30 mice at day 2 post-HI. In contrast, at day 9 post-HI, minocycline-treated P9 mice demonstrated persistent suppression of microglia activation while P30 mice demonstrated a rebound increase in microglial response. P9 minocycline-treated mice demonstrated improved injury at days 2 and 9 post-HI, however no improvement in cerebral atrophy or Morris Water Maze was seen at 60 days post-HI. Conversely, while minocycline treatment did not improve the early injury in P30 mice, these mice did demonstrate significant improvement in cerebral atrophy and Morris Water Maze performance at 60 days post-HI. **Conclusions:** The effect of microglial suppression on HI-induced brain injury varies with age. Neonatal minocycline-treated mice demonstrate an early improvement in injury which is not sustained out to 60 days post-HI, while P30 treated mice demonstrate sustained improvements in cerebral atrophy and memory. This suggests that the late microglial response seen in P30 mice but not P9 mice is neurotrophic and contributes to the observed improvements in cerebral atrophy and neurologic function. Ongoing studies will assess for age-dependent differences in microglia polarization after HI which may account for developmental differences in susceptibility to HI, and the therapeutic effect of suppressing neuroinflammation after injury in the developing brain.


**Poster**

499. Ischemia: Inflammation

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 499.22/I31

**Topic:** C.08. Ischemia
Support: CAPES

CNPq

FUNCAP

Title: Eriodictyol improves memory deficits in pMCAO mice by anti-inflammatory pathways

Authors: A. P. F. M. MENDONCA¹, E. O. FERREIRA¹, N. M. R. LIMA¹, M. Y. S. D. FERNANDES², K. R. T. NEVES², A. A. FONTELES², F. A. V. LIMA², *G. M. ANDRADE³;¹Med. Sci. Dept., ²Physiol. and Pharmacol. Dept., Federal Univ. of Ceara, Fortaleza, Brazil; ³Federal Univ. Ceara, Fortaleza- Ceara, Brazil

Abstract: Cerebral Ischemia (CI) is a common disease and one of the greatest causes of death and disability worldwide. The lack of glucose and oxygen to neuronal tissue leads to a series of events inflammatations culminating in neuronal death. The use of medicinal plants and flavonoids has been widely spread for the treatment of neurodegenerative diseases. Eriodictyol is a flavonoid isolated from the chinese herb Dracocephalum rupestre with anti-inflammatory property proven. Thus, the present study was designed to explore whether eriodictyol has neuroprotective effects against neuronal damage, motor and memory deficits induced by permanent middle cerebral artery occlusion (pMCAO) in mice. Animals were orally treated with eriodictyol (1, 2 and 4 mg/kg) or vehicle (saline) at 30 min before pMCAO, 2 hours after and daily during 3 days. The parameters studied were infarcted brain area, sensorimotor deficit, exploratory activity, working and aversive memory, TNF-α, iNOS and GFAP immunoreactivity and neuronal viability. In vehicle-treated mice, pMCAO resulted in significant cerebral infarction, higher neurological deficit score, decreased exploratory activity and memory deficits. Also were observed an increase on TNF-α and iNOS positive cells and a significant astrogliosis. The treatment with eriodictyol reduced infarct volume, improved the neurological and memory functions, decreased TNF-α, iNOS and GFAP expression and prevented neuronal death ischemic brain injury. Therefore, the results demonstrated neuroprotective effect of the eriodictyol against permanent focal ischemia-induced memory deficits, by a mechanism that involve this anti-inflammatory properties.


Poster

499. Ischemia: Inflammation

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM
Program/#Poster#: 499.23/132

Topic: C.08. Ischemia

Support: Research grant from electroCore LLC

Title: The effect of transcutaneous vagus nerve stimulation on inflammatory markers in acute stroke

Authors: *I. AY¹, B. SIMON², H. AY¹;
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Abstract: Introduction: Cervical vagus nerve stimulation (VNS) reduces tissue injury after middle cerebral artery occlusion (MCAO) in rats. The exact mechanism of the VNS-induced central anti-ischemic effect is unknown. Systemically, VNS attenuates inflammation via activation of the cholinergic system and concomitant inhibition of pro-inflammatory cytokine production. We hypothesized that in ischemic brain VNS-induced neuroprotection is partly mediated via inhibition of pro-inflammatory cytokines. Methods: Right MCAO was induced by 2 hour filament occlusion in spontaneously hypertensive rats. Ipsilateral VNS (bursts of 1 msec duration, 5kHz sine waves repeated at 25 Hz; 2 min long stimulation, delivered at every 10 min for 1 hour) was initiated 30 min after MCAO using a gammaCore, non-invasive nerve stimulator placed on the skin overlying the vagus nerve in the neck. Animals were euthanized either 3 hours or 24 hours after MCAO. Immunohistochemistry was used to assess brain TNF-α, high mobility group box protein 1 (HMGB1), IL-1β, IL-6, and microglial markers Iba1 and CD68 in control (n=4 per time point) and VNS groups (n=4 per time point). In separate cohorts, we investigated the effect of VNS delivered every 15 min either via right (2 min long; n=8) or right/left (1 min long on each side; n=8) stimulation on infarct volume. In control animals (n=8) the right hindlimb was stimulated. Rats were euthanized 7 days later to determine infarct volume. Results: VNS significantly decreased the ischemia-induced increase in TNF-α staining both at 3 hours and 24 hours (p<0.05). VNS significantly increased HMGB1 staining at both time points (p<0.05). In control animals, Iba1-positive cells and CD68-positive cells were detectable at 3 hours and 24 hours after MCAO, respectively. VNS decreased the number of Iba1- and CD68-positive cells at 24 hours after MCAO (p<0.05). Although there were fewer IL-1β and IL-6 positive cells in nVNS-treated animals, the difference was not significant. Animals treated with 15 min-long nVNS had smaller infarct volumes compared with control; reduction was 29% after right nVNS and 26% after right/left nVNS (p<0.05 for both). Conclusion: This study suggests that anti-inflammatory mechanisms via inhibition of TNF-α, HMGB1, and microglia activation play a role in the protective effect of nVNS against ischemic tissue damage.

Disclosures: I. Ay: 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.; electroCore LLC. B. Simon: A. Employment/Salary (full or part-time);; electroCore LLC. H. Ay: None.
Examining cannabinoid neuroprotection, inflammation and hippocampal function in a mouse model of stroke


Abstract: Common to many diseases, including stroke, multiple sclerosis, Alzheimer’s disease and traumatic brain injury, are pathological inflammatory responses leading to neuronal dysfunction and cell death. We have previously demonstrated that cannabinoid receptor 2 agonists reduce infarct size and microglial activation in a mouse model of stroke, indicating neuroprotective effects on the primary infarct as well as on secondary inflammatory responses. To examine the broader impact of secondary inflammation following stroke, we extended our analysis to include behavioral and electrophysiological measures in the hippocampus, a brain region spared by the primary infarct. C57Bl/6 mice were exposed to a focal photothrombotic stroke centered at the primary somatosensory cortex and stroke damage was quantified with tetrazolium chloride staining to determine infarct volume and immunohistochemistry for markers of astrocytes and microglia. Stroked mice showed infarct volumes limited to cortical areas and increased expression of microglia compared to sham surgical controls. In other subjects, hippocampal function was assessed behaviorally two days after the stroke or sham surgery with the spatial object recognition (SOR) test of hippocampal-dependent spatial memory. One to two days after behavioral testing, animals were euthanized and hippocampal slices were prepared for assessment of hippocampal long-term potentiation (LTP), an electrophysiological measure of long-term synaptic plasticity and a cellular correlate of learning and memory. Though the hippocampus was spared by the primary infarct, stroked mice showed both memory impairment in the SOR test and decreased hippocampal LTP. These data indicate that secondary effects of the stroke such as inflammation may produce neuronal damage beyond the infarct zone, impacting hippocampal function. Studies are currently underway to compare these effects of
stroke in wild-type mice to mice with constitutive genetic deletion of cannabinoid receptors and
may highlight the cannabinoid system as a potential therapeutic target in a wide range of
inflammatory disease states.

Disclosures: D.J. Kalamarides: None. P.B. Siegelle: None. K.M. King: None. B.K.
Wellander: None. R.F. Tuma: None. S.J. Ward: None. L.G. Kirby: None.

Poster

499. Ischemia: Inflammation

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#/Poster#: 499.25/I34

Topic: C.08. Ischemia

Support: NNSF 31271142

Title: Shaoyao-gancao decoction ameliorates neurodegeneration in cerebral ischaemia-
reperfusion by inhibiting the inflammation

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Abstract: Stroke rehabilitation is to reduce the neurodegeneration and restore normal life by
regaining and relearning the skills of everyday living, which neurogenesis is the key process.
Inflammation plays an important role in ischemic stroke-reduced neurodegeneration. Controlling
inflammation, ameliorating neuronal death and promoting neurogenesis are important in stroke
rehabilitation. Shaoyao-gancao decoction (SGD) consists of Paeonia lactiflora and Glycyrrhiza
uralensis, a well-known traditional Chinese medicine prescription, is sourced from the Chinese
Medical Classics text_Shanghan Lun, which was used for relieving pain and spasmylosis.
Modern pharmacological studies have demonstrated that the main active ingredient of SGD are
paeoniflorin and liquiritin, which prevents ischemia-induced loss of neuron, and inhibits
activations of microglia and astrocytes in the brain by regulating MAPK and NF-κB signalling
pathway. However, little is known about the potential mechanisms of the SGD for stroke
rehabilitation, which is used in clinic for Chinese patients. To investigate the mechanism of
Shaoyao-gancao decoction in stroke rehabilitation, rats underwent right middle cerebral artery
occlusion for the cerebral ischemia-reperfusion (CI/RP) model. The Bederson scale was used to evaluate behavioral indexes after CI/RP; expression of IL-1β, TNF-α, MCP-1, IL-10, RANTES, VEGF and TGF-β1 were measured by Bio-Plex Magpix System; neurons were stained by Nissl staining and NueN immunohistochemistry staining; Iba1 and GFAP immunohistochemistry staining were used for measuring microglia and astrocytes separately. The results demonstrated that SGD improved the behavioral recovery, increased the number of Neun-positive cells, decreased the number of Iba1- and GFAP-positive cells in brain after CI/RP. These results suggest that Shaoyao-gancao decoction helps stroke rehabilitation by protecting neuron from CI/RP injury, and inhibiting the inflammation.

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Poster

500. Traumatic Brain Injury: Therapeutic Strategies III

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 500.01/135

Topic: C.10. Trauma

Title: Targeting thalamic circuits during deep brain stimulation for traumatic brain injury

Authors: *A. JANSON¹², N. SCHIFF³, J. BAKER³, K. PURPURAZ, J. HENDERSON⁴, B. RUTT⁵, C. R. BUTSON¹²;
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Abstract: Traumatic brain injury (TBI) is a broad term describing an array of complex symptoms and disabilities that can lead to coma or decreased levels of consciousness. Central thalamic deep brain stimulation (DBS) has been demonstrated to modulate arousal in subjects with TBI¹, and the medial dorsal tegmental tract (DTTm) is a specific pathway that has recently been implicated in this response². However, surgical placement of the DBS leads is often guided by anatomical atlases that identify nuclei rather than detailed pathways. The goal of this study is to use computational models to identify DBS lead locations that cause robust modulation of the DTTm. We used computational models to quantify activation of the DTTm using a previously published approach³. The original modeling pipeline was adapted to include segmentation of the central lateral (CL) nucleus of the thalamus using a white-matter-nulled MRI sequence, as well as identification of the DTTm using diffusion weighted MRI followed by tractography.
Activation of DTTm was assessed using a finite element model (FEM) of bipolar stimulation between two adjacent electrode contacts on a Medtronic 3387 lead. Activating function density in the DTTm was measured for a range of lead locations. We identified a gradient of DTTm activation that was strongly dependent on electrode location within the CL (Figure 1). The most robust activation occurred for anterior lead locations where the DTTm fibers converge. We anticipate that analysis of fiber activation and lead location will guide future preoperative planning and postoperative DBS programming to treat TBI. Further, we anticipate that a combination of modeling and *in vivo* testing will elucidate the mechanisms of DBS in ways that were not previously possible.


**Disclosures:** **A. Janson:** None. **N. Schiff:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Dr. Schiff is an inventor of several patents related to neuromodulation therapy.. F. Consulting Fees (e.g., advisory boards); Dr. Schiff has served as a consultant for Intelect Medical. **J. Baker:** None. **K. Purpura:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Dr. Purpura is an inventor of several patents related to neuromodulation therapy.. **J. Henderson:** None. **B. Rutt:** None. **C.R. Butson:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Dr. Butson is an inventor of several patents related to neuromodulation therapy.. F. Consulting Fees (e.g., advisory boards); Dr. Butson has served as a consultant for Intelect Medical, NeuroPace, Advanced Bionics, St. Jude Medical, Boston Scientific and Functional Neuromodulation.

**Poster**

**500. Traumatic Brain Injury: Therapeutic Strategies III**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 500.02/136
**Topic:** C.10. Trauma

**Support:** NIH R01 NS067249

**Title:** Circuit-level modulation of arousal using central thalamic deep brain stimulation

**Authors:** *C. R. BUTSON*\(^1,2\), A. JANSON\(^2\), A. QUINKERT\(^3\), J. BAKER\(^4\), K. PURPURA\(^4\), N. SCHIFF\(^4\), D. PFAFF\(^3\);

\(^1\)Neurol. & Neurosurg., Univ. of Utah, Salt Lake City, UT; \(^2\)Scientific Computing & Imaging (SCI) Inst, Univ. of Utah, Salt Lake City, UT; \(^3\)Rockefeller Univ., New York, NY; \(^4\)Weill Cornell Med. Col., New York, NY

**Abstract:** Deep brain stimulation (DBS) of the central thalamus (CT) has been shown to be promising for the treatment for traumatic brain injury (TBI) (Schiff et al, 2007). However, we do not yet have a detailed understanding of the mechanisms of CT-DBS or the stimulation targets. Previous studies have examined changes in behavior in both mouse and primate models. Bilateral CT-DBS has been performed in both intact (non-injured) and TBI mice (Quinkert & Pfaff, 2012; Quinkert et al, 2010). In tandem with these experiments, non-human primate studies have been conducted to identify neural circuits that robustly modulate arousal during CT-DBS. Studies in both animals have demonstrated that CT-DBS can regulate arousal, and the primate studies suggested that the degree of arousal is modulated by stimulation of the specific fiber pathways including the medial dorsal tegmental tract (DTTm) (Baker et al, submitted). In this study we used computational models to identify circuits that mediate behavioral improvement in the mouse. Behavior was measured using counts (whole body movement, measured as a changing field strength between the subcutaneous transmitter and the receiver beneath the mouse's cage), horizontal activity (fidgeting, all infrared beam breaks in the horizontal plane), and total distance (ambulation, non-repeating beam breaks in the horizontal plane). Stimulation location is a synergistic combination of electrode location, which was determined from post-mortem histology, and stimulation parameters. We used previously published computational modeling methods to predict the volume of tissue activated (VTA) during CT-DBS for each brain hemisphere in each animal (Butson et al, 2007). We then combined the animal-specific models with behavioral outcomes to generate a probabilistic stimulation atlas (PSA) as previously described (Butson et al, 2011). The purpose of the PSA is to identify regions where DBS-induced activation is significantly correlated with changes in behavioral outcome. Lastly, we used the Allen Connectivity Atlas to identify projections that passed through the regions that where stimulation was most strongly correlated with behavioral improvement. We found that those with the largest projection volumes were primary and secondary motor areas, somatosensory cortex and nucleus accumbens. Our results suggest that stimulation location can play an important role in outcomes for CT-DBS, information that may be useful in further development of human therapy for TBI. Further, our approach can enable quantitative comparison of stimulation targets across multiple species including mice, non-human primates and humans.
Disclosures:  C.R. Butson: F. Consulting Fees (e.g., advisory boards); St Jude Medical, Boston Scientific, Functional Neuromodulation.  A. Janson: None.  A. Quinkert: None.  J. Baker: None.  K. Purpura: None.  N. Schiff: None.  D. Pfaff: None.

Poster

500. Traumatic Brain Injury: Therapeutic Strategies III

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 500.03/137

Topic: C.10. Trauma

Support: NIH Grant HD061963

NIH Grant DA031900

Title: Sex-dependent changes in depression and facial allodynia in the chronic period following mild TBI in the mouse

Authors: S. ECKERT, J. SHAW, S. KODURI, A. HERMANN, R. PRASAD, R. ESPANA, *R. RAGHUPATHI;

Abstract: Patients who have sustained a mild TBI suffer from both somatic (headache, dizziness, nausea, fatigue, sleep disturbances) and neuropsychiatric (cognitive deficits, anxiety, depression) symptoms. The effect of gender on response to and outcome after TBI has not received substantive attention both in clinical studies and in pre-clinical animal models. Importantly, a recent study suggested that men and women exhibit different behavioral deficits following sports-related concussions. In the present study, we used a well-established mouse model of mild TBI and assessed facial allodynia and depressive behavior at 4 and 8 weeks post-injury in male and female C57Bl/6 mice. Compared to their sham-injured counterparts, brain-injured male mice exhibited depressive-like behavior (using the forced swim test) at 4 and 8 weeks post-injury (Injury effect, p<0.001); female brain-injured mice were not different from their sham-injured counterparts. To test whether altered dopamine signaling may be the basis for this depression-like behavior, fast scan cyclic voltammetry was used to determine dopamine release kinetics in the nucleus accumbens following stimulation of the ventral tegmental area. Whereas baseline evoked dopamine release was not affected in either brain-injured male or female mice compared to sham-injured mice, cocaine-stimulated dopamine concentration in the nucleus accumbens was greater in the brain-injured male mice compared to their sham-injured counterparts (p<0.01), suggestive of a decreased functionality of the dopamine transporter.
Female brain-injured mice exhibited increased sensitivity to periorbital stimulation (using the von Frey filament test) compared to sham-injured mice at both 4 and 8 weeks post-injury (injury effect, p<0.001), suggestive of post-traumatic headache; male brain-injured mice were not different from their sham-injured counterparts. Together, these data are indicative of sex-dependent differences in the response to mild TBI and underscore the importance of evaluation of behavioral and biochemical measures in the chronic post-traumatic period.


Poster

500. Traumatic Brain Injury: Therapeutic Strategies III

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 500.04/138

Topic: C.10. Trauma

Support: CHIR to DE

Title: Determining mean heart rate at symptomatic threshold in post-concussion syndrome

Authors: *M. LETOURNEAU¹, C. ALARIE²; D. MOORE², D. ELLEMBERG²; ¹Kinesiology, Neurodevlab, Univ. of Montreal, Montreal, QC, Canada; ²Kinesiology, Univ. of Montreal, Montreal, QC, Canada

Abstract: Recent research demonstrates that aerobic exercise protocols are promising for rehabilitating individuals suffering from post-concussion syndrome. However, exercise protocols usually include the frequent triggering/exacerbating of symptoms in order to track progress and adjust exercise intensity. Unfortunately, triggering/exacerbating symptoms can impede recovery and deter adherence. Therefore, we sought to provide a less traumatic, more efficacious and therapeutic use of exercise by establishing the threshold at which symptoms are triggered/exacerbated. Doing so will enable the avoidance of exercise induced symptoms and allow the personalization of aerobic exercise protocols. Eighteen concussed individuals (25.8yrs, ± 10.2) reporting persisting symptoms at rest (m=91.6 ± 78.0 days) completed a graded exercise test (GXT) on a stationary bike until symptoms were triggered/exacerbated. Participants had a mean resting heart rate of 75.7 bpm (± 9.0). We observed that symptoms were triggered/exacerbated at a mean heart rate of 123.6 BPM (± 23.7), which corresponded to 42.0% (± 18.95) of the heart rate reserve and 65.5% (± 12.2) of maximal theoretical heart rate. Interestingly, neither age at injury nor number of injuries was correlated with symptomatic
threshold \((p > 0.05)\). These results help determine the symptom threshold in concussed individuals, which can be used to implement a less traumatic exercise protocol, while enhancing rehabilitative efficacy by not voluntarily triggering/exacerbating symptoms. Thus, our data suggest that individuals experiencing persistent symptoms after a concussion would benefit from initiating physical activity by starting at an intensity below aforementioned symptomatic thresholds.

**Disclosures:**  M. Letourneau: None. C. Alarie: None. D. Moore: None. D. Ellemberg: None.

**Poster**

**500. Traumatic Brain Injury: Therapeutic Strategies III**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 500.05/I39

**Topic:** C.10. Trauma

**Title:** Delayed thymosin beta 4 treatment improves functional recovery via neurovascular remodeling in rats after traumatic brain injury

**Authors:** *Y. XIONG*1, Y. ZHANG1, Y. MENG1, Z. LIU2, D. C. MORRIS1, Z. G. ZHANG2, A. MAHMOOD1, M. CHOPP2,4; 1Neurosurg., 2Neurol., 3Emergency Med., Henry Ford Hlth. Syst., Detroit, MI; 4Dept. of Physics, Oakland Univ., Rochester, MI

**Abstract:** Object. Our previous studies demonstrate that thymosin beta 4 (Tβ4) significantly improves functional recovery in rats after traumatic brain injury (TBI). Here, we investigated potential mechanisms underlying the Tβ4 therapeutic effect in rats after TBI. Methods. Adult male rats subjected to TBI induced by controlled cortical impact received saline or Tβ4 (6 mg/kg, RegeneRx Biopharmaceuticals Inc, Bethesda, MD) ip starting 24 hr post injury and then every third day for 2 weeks. We evaluated functional outcome after different treatments. We injected biotinylated dextran amine before injury into the contralateral intact cortex for anterogradely labeling corticospinal track (CST). Animals were sacrificed at 35 days post injury. Their spinal cords were processed for measurement of midline-crossing axons. Immunostaining with brain sections was performed for assay of angiogenesis and neurogenesis. A set of rats were sacrificed at 2 days after TBI for assay of Angiopoietin 1 (Ang1), Ang1 receptor Tie2, Akt, and cAMP responsive element-binding protein (CREB) expression. Results. Tβ4 treatment significantly improved spatial learning and sensorimotor functional recovery, promoted angiogenesis and neurogenesis, as well as increased expression of Ang1, Tie2, p-Akt, p-CREB in the injured cortex and hippocampus compared to saline treatment \((p<0.05)\). Tβ4 administration
significantly promoted axonal sprouting from the intact side into the denervated side in both the cervical and lumbar enlargements in TBI rats compared to saline controls (p<0.05). The number of axons crossing at the cervical (p<0.001) and lumbar (p<0.001) spinal cord was highly and inversely correlated to the incidence of forelimb and hindlimb footfaults examined at Day 35 after TBI. The total number of axons crossing was highly and inversely correlated to the mNSS score assessed at Day 35 after TBI (p<0.05). Conclusions. Our data indicate that promotion of neurovascular remodeling through activation of Ang1/Tie2/Akt signaling pathway may contribute to the therapeutic effect of Tβ4 after TBI.


Poster

500. Traumatic Brain Injury: Therapeutic Strategies III

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 500.06/140

Topic: C.10. Trauma

Title: Deletion of aquaporin-4 is neuroprotective during the acute stage of micro traumatic brain injury in mice

Authors: *Z. PEI1, F. LIANG1,2, C. LUO2;
1The First Affiliated Hospital, Sun Yat-Sen Univ., Guangzhou, Guangdong, China; 2State Key Lab. of Quality Res. in Chinese Medicine, Inst. of Chinese Med. Sciences, Univ. of Macau, Macau, China

Abstract: Micro traumatic brain injury (TBI) is the most common type of brain injury, but the mechanisms underlying it are poorly understood. Aquaporin-4 (AQP4) is a water channel expressed in astrocyte end-feet, which plays an important role in brain edema. However, little is known about the role of AQP4 in micro TBI. Here, we examined the role of AQP4 in the pathogenesis of micro TBI in a closed-skull brain injury model, using two-photon microscopy. Our results indicate that AQP4 deletion reduced cell death, water content, astrocyte swelling and lesion size during the acute stage of micro TBI. Our data revealed that astrocyte swelling is a decisive pathophysiological factor in the acute phase of this form of micro brain injury. Thus, treatments that inhibit AQP4 could be used as a neuroprotective strategy for micro TBI.

Disclosures: Z. Pei: None. F. Liang: None. C. Luo: None.
**Poster**

500. Traumatic Brain Injury: Therapeutic Strategies III

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 500.07/141

**Topic:** C.10. Trauma

**Title:** The use of the dig task to explore the effectiveness of magnesium on recovery of function after traumatic brain injury

**Authors:** *J. YOUNG, M. R. HOANE;
Psychology: Brain and Cognitive Sci., Southern Illinois Univ., Carbondale, IL

**Abstract:** Currently, an effective pharmacological treatment after traumatic brain injury (TBI) that returns a patient to a normal level of functioning is nonexistent. In order to better examine the effectiveness of a drug treatment after TBI, tasks that assess all aspects of cognitive function are needed. Discrimination tasks, such as the dig task, can be used to examine cognitive deficits after an experimental TBI in rodents. The dig task is a two-choice scent discrimination task that can effectively assess the cognitive abilities of the rat after a TBI has been induced. The task can be used to determine if the pharmacological treatment is improving the rat. A pharmacological treatment of magnesium has been previously shown in other studies to be a viable treatment for the recovery of cognitive and motor function after TBI. Magnesium plays an important role in the pathophysiological processes after insult and can help to promote cognitive function if delivered correctly. For this reason, a study will be conducted in order to further evaluate the effectiveness of magnesium after a TBI using the dig task. 30 male Sprague Dawley (Harlan, Indianapolis, IN) will be used and separated into either MAG/TBI, VEH/TBI, or VEH/Sham groups. Before induction of a bilateral frontal injury, rats will be pre-trained on the dig task as well as the locomotor placing task. After surgery, rats will receive either an intraperitoneal injection of 2 mmol/kg magnesium chloride or 0.1% phosphate buffer solution (PBS). Magnesium injections will occur 4 hours post-surgery then at 24 hours and again at 72 hours. After 10 days of recovery rats will begin testing on the dig task. The dig task includes two scent pairings; basil (baited) versus coffee, then the reversal, cocoa (baited) versus cumin, and then the reversal. The locomotor placing task will also be conducted in order to assess for the recovery of motor function after TBI. Ideally the results of this study will demonstrate that magnesium is an effective treatment after a traumatic brain injury. It is expected that the dig task will assess acute and prolonged cognitive deficits following the frontal brain injury. It is also expected that the locomotor placing task will assess for any motor deficits.

**Disclosures:** J. Young: None. M.R. Hoane: None.
**Poster**

**500. Traumatic Brain Injury: Therapeutic Strategies III**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 500.08/142

**Topic:** C.10. Trauma

**Support:** NIH Grant 5R25GM102776

**Title:** Restoration of enzymatic activity of energy related proteins in traumatically brain injured rats following the administration of gamma-glutamylcysteine ethyl ester

**Authors:** *T. T. REED, B. B. RICE;* Eastern Kentucky Univ., Richmond, KY

**Abstract:** Biochemical processes such as the glycolytic pathway and Kreb’s cycle are important in producing ATP for the brain. Without a sufficient supply of glucose for energy metabolism, the brain cannot efficiently regulate or coordinate the actions and reactions of the body. The disruption of brain function resulting from an external force, such as a bump, is known as traumatic brain injury (TBI). Symptoms of TBI range from physical to psychological while effects are indicative of the severity of injury experienced. TBI is associated with reduced energy metabolism, as studies have demonstrated that protein nitration is consequential of TBI through the production of reactive oxygen/nitrogen species (ROS/RNS). Antioxidants, such as glutathione (GSH), combat the deleterious effects of oxidation by scavenging ROS/RNS, inhibiting propagation, and removing neurotoxic byproducts. Gamma-glutamylcysteine ethyl ester (GCEE) is an ethyl ester moiety of gamma-glutamylcysteine that exhibits antioxidant activity by increasing GSH production. Previous studies have demonstrated that the administration of GCEE following TBI has protective effects against protein nitration through the elevation of glutathione. This study investigates the enzymatic activity of energy related proteins that have been identified as nitrated in moderate TBI treated Wistar rats. To test the hypothesis that the elevation of GSH production upon administration of GCEE will normalize enzymatic activity post-TBI, adult male Wistar rats were equally divided into three groups: sham, saline, and GCEE. Rats in all groups (except sham) were subjected to a craniotomy and a moderate TBI via cortical contusion. Post-TBI rats treated with saline or GCEE groups received 150 mg/kg of saline and GCEE, respectively. Upon sacrifice, brains were harvested and enzymatic activity was measured spectrophotometrically. Preliminary results demonstrate an increase in enzymatic activity upon GSH elevation via GCEE administration in several key enzymes, thereby indicating GCEE is a potential therapeutic strategy to restore energy related proteins in the brain post-TBI via GSH elevation.
Traumatic brain injury (TBI) results in important neurological impairments which occur through a cascade of deleterious physiological events over time. No effective treatment exists to prevent these consequences. Etifoxine (EFX) is a non-benzodiazepine compound exhibiting anxiolytic properties in the treatment of adjustment disorders with anxiety (1). An enhancement of GABAergic neurotransmission underlies its anxiolytic profile directly after binding on the GABA_A receptor, or indirectly, involving the activation of translocator protein 18 kDa (TSPO) that leads to an increase in the synthesis of neuroactive steroids (2). EFX displays potent regenerative and anti-inflammatory properties, promotes functional recovery in experimental models of traumatic peripheral nerve injury, and reduces brain edema in rats (3-5).

TBI is followed by a profound reorganization of the GABAergic system, a dysregulation of TSPO levels (6), and a dramatic inflammatory response characterized by the release of pro- and anti-inflammatory cytokines. In previous studies, we studied the effects of EFX treatment in a model of mild TBI induced by controlled cortical injury (CCI) in rats, and showed that EFX treatment improved sensorimotor recovery (7). In the present study, we assessed the effect of EFX on CCI-induced neuroinflammation. Male SD rats were subjects to sham surgery or CCI and sacrificed at 6 hours. EFX (50 mg/kg, i.p.) or vehicle was administered 30 minutes after injury. We measured several cytokines levels in the ipsi and contralateral cerebral cortex of rats and looked at the effects of EFX treatment on inflammation markers. Compared to injured vehicle treated rats, EFX treatment reduced pro-inflammatory cytokines levels, without affecting anti-inflammatory cytokines levels. Altogether, these results suggest that in mild TBI CCI model, post injury treatment with EFX is effective in reducing neuroinflammation and improves functional recovery. These findings suggest that EFX may have a therapeutic potential in the treatment of TBI. The effect of EFX on neuronal death, astrocytes and microglia activation is currently being investigated in our model. 1. Nguyen et al., *Hum Psychopharmacol*. 21, (2006).

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**Disclosures:** E. Simon O'Brien: A. Employment/Salary (full or part-time); Biocodex. D. Gauthier: A. Employment/Salary (full or part-time); Biocodex. V. Riban: A. Employment/Salary (full or part-time); Biocodex. M. Verleye: A. Employment/Salary (full or part-time); Biocodex.

**Poster**

500. Traumatic Brain Injury: Therapeutic Strategies III

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 500.10/144

**Topic:** C.10. Trauma

**Support:** NIH/NINDS F31-NS08639

NIH/NINDS T32-NS077889

NIH/NINDS R01-NS048191

NIH/NINDS R01-NS062993

**Title:** A “neet” target for traumatic brain injury: pioglitazone and mitoneet interactions improve tbi related mitochondrial dysfunction

**Authors:** *H. M. Yonutas*¹, J. Pandya², A. Sebastian², W. Geldenhuys³, R. Carroll³, P. G. Sullivan¹;


**Abstract:** Traumatic Brain Injury (TBI) is difficult to treat due to the complicated secondary injury cascade that is activated post-injury. The most promising therapeutics are multi-targeted, improving neuroinflammation, ROS production and mitochondrial dysfunction. Pioglitazone, an
FDA approved drug for Type 2 Diabetes and a known PPAR agonist, has shown promise in altering neuroinflammation and decreasing ROS production. Work from our lab found that pioglitazone can increase mitochondrial bioenergetics within the first 12 hours of injury leading to improved cortical sparing and functional recovery. This effect seems to be too rapid for PPAR activation alone and may be due to its ability to bind a novel mitochondrial protein called mitoNEET. We hypothesize that targeting mitoNEET with a mitoNEET ligand, such as pioglitazone, leads to improved tissue and functional recovery. To test this hypothesis we used a severe Controlled Cortical Impact (CCI) injury model, mitoNEET null and wild-type mice, pioglitazone and a novel mitoNEET ligand called NL-1, which has a similar structure to pioglitazone however contains no PPAR binding region. In a simulated excitotoxicity experiment, ex vivo mitochondrial studies show that pioglitazone and NL-1 can increase bioenergetics in isolated cortical mitochondria with and without Ca2+ insult. An in-vivo pioglitazone and NL-1 dose-response study was then preformed in wild-type mice post-injury. The dosages which provided the greatest amelioration of mitochondrial dysfunction were used in wild-type and mitoNEET null mice subjected to sham or severe CCI surgery. Pioglitazone lost its ability to increase mitochondrial respiration and provide neuroprotection in mitoNEET null mice. Additionally, the mitoNEET specific ligand, NL-1, increased cortical sparing and functional recovery and decreased MRI T2-weighted hyperintensity at the injury site. These results support the role of mitoNEET in the neuropathological sequelae of brain injury and as a crucial target for pioglitazone mediated neuroprotection following TBI.


Poster

500. Traumatic Brain Injury: Therapeutic Strategies III

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 500.11/145

Topic: C.10. Trauma

Support: Supported by award W81XWH-10-2-0171 from the USAMRMC to P.J.B

Title: Minocycline plus N-acetylcysteine have a clinically useful therapeutic window in two animal models of traumatic brain injury

Authors: *M. A. SANGOBOWALE, N. M. GRIN’KINA, K. WHITNEY, P. J. BERGOLD; Pharmacol. and Physiol., SUNY Downstate Med. Ctr., Brooklyn, NY
Abstract: There are presently no drugs to treat traumatic brain injury (TBI). We have previously shown that the combination of the FDA-approved drugs minocycline (MINO) and N-acetylcysteine (NAC) synergistically improved cognition and memory, modulated inflammation, limited grey matter injury and induced remyelination when dosed 1 hour after injury in a rat controlled cortical impact model (CCI) of TBI. MINO plus NAC retained similar efficacy in a mouse closed head injury (CHI) model of TBI. The CHI model was then used to determine the lowest dose of both drugs that retained full efficacy. When dosed at one hour after CCI or CHI, the optimized MINO plus NAC dose modulated neuroinflammation and induced remyelination in the injured brain. We now report that this optimized dose of MINO plus NAC improved cognition and memory when dosed 6 hours after injury in the rat CCI and mouse CHI models. This therapeutic window was assessed using an active place avoidance task with high cognitive demand. MINO plus NAC, however, no longer improved cognition in the rat CCI model when dosed 12 hours after injury. We are testing whether the 6-hour therapeutic window of MINO plus NAC may be longer when tested using Barnes maze, a behavioral task that likely has a lower cognitive demand than active place avoidance. We are also testing whether the 6-hour dosing of MINO plus NAC modulates inflammation, limits grey matter injury and induces remyelination. These data suggest that MINO plus NAC limits brain injury with a clinically useful therapeutic window in two TBI models and in two species. These preclinical studies provide further evidence that that MINO plus NAC has sufficient potency and safety to be tested against clinical TBI.

Disclosures: M.A. Sangobowale: None. N.M. Grin’kina: None. K. Whitney: None. P.J. Bergold: None.

Poster

500. Traumatic Brain Injury: Therapeutic Strategies III

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 500.12/146

Topic: C.10. Trauma

Support: Craig H. Neilsen 313739

  NIH R01NS067092

  NIH R01NS088475

  Veterans Affairs Research Enhancement Award (RAS)
Title: Multi-modal interventions for improving recovery after TBI assessed by a data-driven multivariate approach

Authors: *J. HAEFELI1, A. R. FERGUSON1, D. BINGHAM2, A. ORR2, S. WON2,1, T. I. LAM2,1, J. SHI2,1, S. HAWLEY2, J. LIU2,1, R. A. SWANSON2,1, S. M. MASSA2,1;
1UCSF, San Francisco, CA; 2San Francisco Veterans Affairs Med. Ctr., San Francisco, CA

Abstract: Traumatic brain injury (TBI) causes a complex cascade of events involving primary cell death, secondary injury, and chronic inflammation, all of which work in concert to impact recovery, leading to a highly complex syndrome. Due to growing evidence that combinatorial therapies might have cumulative effects, a combination of neuroprotective drug interventions including minocycline and LM11A-31 (a small molecule p75 neurotrophin receptors modulator), and physical therapy (i.e., conventional or botulinum toxin-induced constraint movement therapy) were applied in a controlled cortical impact (CCI) TBI model in rats. To remain sensitive to multimodal changes induced by the combinatorial interventions, we took a data-driven multivariate approach to leverage the full set of outcome measures ranging from cellular to long-term motor and cognitive behavior. The interaction effects (i.e., synergistic and counteracting) of different drug interventions and physical therapies were assessed using a linked analytical workflow involving principal component analysis followed by a linear mixed model (PCA-LMM). The linear mixed model was used to test the effect of drug intervention and physical therapy on principal components scores extracted from functional outcome measures and brain markers. This analytical workflow was applied to a prospectively collected database including 202 animals (79 shams, 123 TBI animals). Both sham and TBI rats received minocycline, LM11A-31, physical therapy and constraint-induced movement therapy delivered alone or in combination. Animals were assessed with an extensive functional outcome battery (i.e., forelimb use asymmetry, vermicelli handling, Morris water maze and sticky label tests) and cortical lesion volume and glial activation were evaluated. Results revealed a significant benefit of LM11A-31 on multidimensional recovery after TBI (F=14.05, p<0.001). This effect depended on the type of physical therapy intervention applied (F=9.77, p<0.001). Further, LM11A-31 and minocycline had a synergistic effect on multidimensional learning and memory outcomes (F=5.41, p=0.022). This data provides evidence of combinatorial effects of drug and physical therapy interventions on multidimensional outcomes following experimental TBI in a large-scale dataset.

**Poster**

**500. Traumatic Brain Injury: Therapeutic Strategies III**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 500.13/147

**Topic:** C.10. Trauma

**Title:** effect of aspirin and clopidogrel on bleeding, platelet aggregation, and neuronal damage following traumatic brain injury

**Authors:** *F. H. KOBEISSY*¹, M. NASSER², D. SERHAN³, F. DAKROUB², Z. DALLOUL², E. HAMADE⁴, K. ZIBARA⁵, H. DARWICHE²; ¹Dept of Psychiatry, Univ. of Florida, Gainesville, FL; ²Chemistry, ³American Univ. of Beirut, Beirut, Lebanon; ⁴Lebanese Univ., Beirut, Lebanon

**Abstract:** Background: Traumatic brain injury (TBI) often referred to as the “silent epidemic,” is a non-degenerative non congenital insult to the brain due to a blow or penetrating object that disrupts the function of the brain leading to permanent or temporary impairment of cognition, physical and psychosocial functions. Antiplatelet agents such as clopidogrel and Acetyl-Salicyclic Acid- ASA (aspirin) inhibits the formation of blood clot by inhibiting platelet aggregation and activation and are essential adjuncts to the medical care of patients with cardiovascular disease and had been co-administered in elderly patients. Clinical studies show that patients taking anti platelets before head trauma are at an increased risk to develop serious intra cranial hemorrhage when compared to untreated patients. Aims: In this study, we will assess the different aspects of systemic changes (bleeding, platelets activation and thromboxane levels) as well as different bio-markers of brain injury after co-administration of clopidogrel and aspirin after experimental model of brain injury and in control group. Methods: Rats were divided into five groups (control, TBI , TBI +Aspirin 20 mg/kg ip , TBI+ clopidogrel 10 mg / kg , TBI +Combination) treatments were given for 2 days and sacrifice was done 48 hours post injury. Western blotting was performed on brain samples to assess the changes in the levels of different proteins (spectrin, Gfap and transferrin). Immunofluorescence (IF) was also performed on different proteins notably Gfap and Neun. Finally, ELISA and EIA were used to find relevant serum proteins associated to brain injury and inflammation (IL6 , IL10 , tnfr and thromboxane ). Results: Aspirin and Clopidogrel inhibited platelet aggregation alone and in combination also Thromboxane (TXB2) whose level is indicative of activated platelets, increased after TBI when compared to control group however its level in all treated groups was comparable to control. WB data confirmed that spectrin cleavage (an early marker of necrotic and apoptotic cell death
following TBI) increased in TBI when compared to control groups. and in rats treated with combination, spectrin cleavage is highest among all groups.

**Disclosures:** F.H. Kobeissy: None. M. Nasser: None. D. Serhan: None. F. Dakroub: None. Z. Dalloul: None. E. Hamade: None. K. Zibara: None. H. Darwiche: None.

**Poster**

500. Traumatic Brain Injury: Therapeutic Strategies III

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 500.14/48

**Topic:** C.10. Trauma

**Support:** Funds from BARDA, OASPR, DHHS, under contract with Countervail Corp. [Contract HHSO100201100030C]

**Title:** Galantamine as an effective co-adjuvant medical countermeasure to counter the delayed neurotoxic effects of the organophosphorus compound sarin

**Authors:** *E. F. PEREIRA¹, Y. ARACAVA¹, J. D. PESCRILLE¹, D. CARTER¹, L. RICHARDSON¹, D. MCKOY¹, L. MCCOWAN¹, E. ALEXANDROVA¹, N. PHAM¹, E. LUMSDEN¹, R. CLARK¹, J. MAMCZARZ², S. XU¹, R. P. GULLAPALLI¹, M. LANE¹, I. MERCENTHALER¹, G. W. BASINGER, Jr.², E. X. ALBUQUERQUE¹;

¹Div. of Translational Toxicology, Dept Epidemiol Publ Hlth., Univ. Maryland Sch. Med., Baltimore, MD; ²Countervail Corp, Charlotte, NC

**Abstract:** The acute toxicity of OP compounds, including the nerve agent sarin, results primarily from the irreversible inhibition of acetylcholinesterase (AChE). While conventional antidotal therapy consisting of atropine (to block overactivation of muscarinic receptors), oximes (e.g. 2-PAM, to reactivate OP-inhibited AChE), and benzodiazepines (to halt convulsions) helps mitigate the acute signs of OP intoxication, they do not adequately prevent long-term neurotoxic effects [J Pharmacol Exp Ther 350: 313-321, 2014]. Here, we used a multi-disciplinary translational approach to: (i) assess the delayed neurotoxicity of 1.2xLD50 sarin (108 µg/kg, sc) in rats treated with atropine (0.5 mg/kg, im)-plus-2-PAM (25 mg/kg, im) administered 1 min post-sarin with or without diazepam (0.72 mg/kg, im, at onset or 20 min after onset of convulsions), and (ii) evaluate the benefits of using galantamine (0.3-3.0 mg/kg, im, 6 h post-sarin) as a co-adjuvant therapy. Between 3 and 5 months after the exposure to sarin, rats treated with atropine-plus-2-PAM with or without diazepam, administered 20 min post-onset of convulsions, presented significant spatial learning and memory deficits in the Morris water maze.
Between 2 and 6 months after the injection of sarin, the total EEG power and the power of individual EEG frequencies in these rats were also higher-than-normal. Diazepam given at onset of convulsions to sarin-exposed rats prevented the learning impairment and the changes in EEG activity, but not the memory deficits. *In vivo* imaging revealed that, between 2 and 6 months after the injection of sarin followed by treatment with atropine-plus-2-PAM, diazepam (at onset or 20 min after onset of convulsions), rats presented significant metabolic and structural alterations in areas of the brain that are known to play an important role in controlling cognitive function, including the hippocampus, thalamus, and striatum. Finally, immunohistochemistry revealed significant and persistent neuronal loss in the hippocampus and lateral amygdala of sarin-injected rats that were post-treated with atropine-plus-2-PAM with or without diazepam (administered at onset or 20 min after onset of convulsions). Inclusion of galantamine in the post-treatment regimen effectively countered the behavioral deficits as well as the electrical and structural alterations in the brain of sarin-exposed rats, with the most effective dose of galantamine being 3 mg/kg. In conclusion, using various metrics, the present study demonstrates the beneficial effects of the use of galantamine as a co-adjuvant medical countermeasure to mitigate the delayed neurotoxic effects of OP compounds.


**Poster**

**500. Traumatic Brain Injury: Therapeutic Strategies III**

**Location**: Hall A

**Time**: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 500.15/J1

**Topic**: C.10. Trauma

**Support**: NIH CounterAct Program through the NINDS award R44 NS068049

**Title**: Efficacy of oral galantamine pre-treatment against soman toxicity in guinea pigs

**Authors**: *Y. ARACAVA*¹, J. D. PESCRILLE¹, D. CARTER¹, R. CLARK¹, L. RICHARDSON¹, M. LANE¹, E. F. PEREIRA¹, E. X. ALBUQUERQUE¹, G. W. BASINGER, Jr.²;

¹Div. of Translational Toxicology, Dept Epidemiol Publ. Hlth., Univ. Maryland Sch. Med., Baltimore, MD; ²Countervail Corp, Charlotte, NC
Abstract: Previous studies from several laboratories have assessed the effectiveness of numerous medical countermeasures as pre-treatments against the acute toxicity of nerve agents [e.g., PNAS 103:13220, 2006; Neurotoxicology 23:1, 2002]. Because absorption of a drug delivered intramuscularly, particularly to laboratory animals, is more consistent than the absorption of drugs delivered orally, the preferred route of administration of the medical countermeasures in the majority of those studies was the intramuscular route. Yet, the variety of dose forms, in addition to the convenience and safety of use, make the oral route the preferred route for human use. For example, pyridostigmine has been approved by the FDA for oral use by the military personnel at risk of exposure to soman [Neurologist 13:20, 2007]. In recent years, galantamine emerged as an effective medical countermeasure against OP poisoning. Thus, the present work was designed to determine the effectiveness of orally delivered galantamine as a pre-treatment to reduce the acute toxicity and lethality induced by soman in guinea pigs. To this end, male and female guinea pigs were treated orally with galantamine HBr (8, 10, or 12 mg/kg), pyridostigmine HBr (1.9 mg/kg), or vehicle and injected 30 min later with 1.0x or 2.0xLD50 soman (1.0xLD50 = 28 µg/kg). Animals that were injected with 2.0xLD50 soman were also treated 1 min later with atropine (0.5 mg/kg, im)-plus-2-PAM (25 mg/kg, im) and 5 min after onset of convulsions with midazolam (2.8 mg/kg, im). Survival was significantly greater among soman (1.0xLD50)-challenged guinea pigs pre-treated orally with galantamine (8, 10, or 12 mg/kg) than among those pre-treated with pyridostigmine or saline. In male and female guinea pigs challenged with 2.0xLD50 soman, pre-treatment with 12 mg/kg galantamine in association with post-treatment with conventional antidotes afforded significantly greater survival than conventional antidotes in association with pre-treatment consisting of pyridostigmine or vehicle. Oral pre-treatment with galantamine was also significantly superior to pre-treatment with pyridostigmine in reducing neuronal cell death in the brains of guinea pigs challenged with 2.0xLD50 soman and post-treated with standard care and in the brains of guinea pigs challenged with 1.0xLD50 soman and provided with no additional supportive post-treatment. Compared to saline and pyridostigmine, galantamine reduced by more than 25% the neuropathology in the brain of soman-injected guinea pigs. In conclusion, oral galantamine emerged as a better pre-treatment than oral pyridostigmine in countering the acute toxicity of soman.

Program#/Poster#: 500.16/12

Topic: C.10. Trauma

Support: NIH CounterAct Program through the NINDS award R44 NS068049

Title: Pharmacokinetics of galantamine following oral administration to guinea pigs

Authors: W. P. FAWCETT¹, R. H. COOMBES¹, Y. ARACAVA¹, J. D. PESCRILLE¹, G. W. BASINGER, Jr.², E. F. PEREIRA¹, *E. X. ALBUQUERQUE¹;
¹Div. of Translational Toxicology, Dept Epidemiology and Publ. Hlth., Univ. Maryland Sch. Med., Baltimore, MD; ²Countervail Corp, Charlotte, NC

Abstract: Galantamine, a drug approved to treat Alzheimer’s disease, has emerged as an effective medical countermeasure against organophosphorus (OP) intoxication [PNAS 103:13220-13225, 2006]. Here, we examined the pharmacokinetic profile of galantamine following its oral administration to guinea pigs and the corresponding degree of blood AChE inhibition. To this end, galantamine was dissolved in pharmaceutical grade saline (0.9%) and an adequate volume of the solution was administered together with an equal volume of a vegetable juice to the animals. A 1-ml disposable syringe was used to deliver orally 8, 10, or 12 mg/kg galantamine to the animals. Plasma galantamine levels were measured using an HPLC coupled to a UV detector, and blood AChE activity was measured using a radiometric assay. Time to reach maximal plasma concentration was approximately 1 h, indicating that in guinea pigs, as in humans, oral galantamine is quickly absorbed. Maximal plasma concentrations of galantamine (Cmax) and area-under-the-curve of plasma concentrations of galantamine over time (AUC) were linearly correlated with the test doses. The degree of blood AChE inhibition changed with time following oral administration of galantamine HBr to guinea pigs as anticipated by the pharmacokinetic profile of the drug. Maximal degree of AChE inhibition (Imax) and the area-under-the-curve of AChE inhibition over time were significantly correlated with Cmax and area-under-the-curve of plasma concentrations of the drug over time. The pharmacokinetics of orally administered galantamine and the degree of AChE inhibition produced by each dose were comparable between males and females. The absolute oral bioavailability of galantamine in male and female guinea pigs was found to be approximately 50%. The lower bioavailability of galantamine in rodents than in humans may be accounted for by species-specific metabolism. Although rodent models can be used to predict oral drug absorption in humans, they are generally poor predictors of drug metabolism or oral bioavailability in humans [Pharm Res 23:1675-1686, 2006]. The maximal degrees of AChE inhibition attained following oral treatment of guinea pigs with the test doses of 8, 10, and 12 mg/kg galantamine HBr ranged from 40% to 55%, which are within the range of AChE inhibition measured in the blood of humans treated with approved doses of the drug [Clin Pharmacol Ther 50: 420-428, 1991]. In conclusion, the pharmacokinetic profile and the degree of AChE inhibition resulting from oral treatment of guinea pigs with doses of
galantamine in the range of 8 to 12 mg/kg indicate that these doses of galantamine will convert
to oral doses that are safe for human use.


Poster
500. Traumatic Brain Injury: Therapeutic Strategies III

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 500.17/J3

Topic: C.10. Trauma

Support: The Ari and Regine Aprijaskis Fund, at Tel-Aviv University

Title: Hyperbaric oxygen therapy as a potential treatment for traumatic brain injury

Authors: *C. G. PICK¹, R. BARATZ-GOLDSTEIN¹, V. RUBOVITCH², S. TOUSSIA-COHEN²;
²Anat., ¹Tel Aviv Univ., Tel Aviv, Israel

Abstract: Introduction: Traumatic brain injury is a common health problem with significant
effect on quality of life. Hence, TBI is a major social problem and economic burden. The major
causes are motor vehicle crashes, falls, and violence. Mild traumatic brain injury (TBI) accounts
for 80-90% of total brain injuries. mTBI may lead to short- and long-term cognitive, emotional,
and behavioral deficits. As yet, there is no effective treatment or cure for patients with mTBI.

Hyperbaric oxygen therapy (HBOT) is a treatment by which 100% oxygen is administered at a
pressure greater than atmospheric pressure at sea level (one atmosphere absolute, ATA). This
involves placing the patient in an airtight vessel, increasing the pressure within that vessel, and
administering 100% oxygen for respiration. In this way, it is possible to deliver a greatly
increased partial pressure of oxygen to the tissues. HBOT has been shown to decrease cerebral
edema, normalize water content in the brain, decrease the severity of brain infarction, and
maintain blood-brain barrier integrity.

Methods and Results: Mice were subjected to closed head
weight-drop injury with 70 g weight. Mice were treated with hyperbaric oxygen for 1 hour at 2
ATA for 4 consecutive days starting from 3 hours post injury. 7 days post injury mice were
assessed in 2 behavioral paradigms: Y-Maze and Novel Object Recognition test. Mice exhibited
a lower learning ability following mTBI in both the Y-Maze and Novel Object Recognition test.
The cognitive impairments were ameliorated in mice treated with HBOT. Brains (from another
group) were remove 72 hours post last HBO treatment. mTBI group had decrease in myelin basic
Furthermore, we found increase in neuronal loss and in astrocyte reactivity post brain injury. These changes were abolished in mice that were treated with HBOT. Conclusions: These findings may suggest a new therapeutic strategy to treat damages induced by mTBI. The mechanisms underlie this improvement may be related to reducing inflammation and preventing de-meyelination.

Disclosures:  C.G. Pick: None. R. Baratz-Goldstein: None. V. Rubovitch: None. S. Toussia-Cohen: None.

Poster

500. Traumatic Brain Injury: Therapeutic Strategies III

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 500.18/34

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant R01 NS062097

NIH Grant R01 NS058710

NIH Grant R01 NS085568

Title: Wnt3a protects against autophagic cell death after traumatic brain injury

Authors: *J. Y. ZHANG, J. LEE, S. P. YU, L. WEI;
Emory Univ., Decatur, GA

Abstract: Traumatic brain injury (TBI) is the leading cause of morbidity and mortality in children and young adults, including soldiers and athletes. However, there are currently no effective pharmacological therapies for TBI. Wnt proteins are a family of signaling ligands that are critical for many aspects of development, including cell division, differentiation, and migration, cell fate specification, and axis formation. In adults, Wnt ligands still play major roles in multiple organ systems. In the adult brain, canonical Wnt ligands, such as Wnt3a, mediate endogenous neural stem cell (NSC) proliferation and differentiation, which is an important component of tissue repair. We expected that supplementation of recombinant Wnt3a following TBI would increase endogenous neurogenesis. While our previously reported results supported our original hypothesis, we now report on the novel discovery of a protective effect of Wnt3a that acts through a mechanism independent of neurogenesis. Specifically, Wnt3a is able to directly attenuate autophagic programmed cell death that results from TBI. The whisker barrel pathway provides a useful serial system for studying both the functional deficits and recovery
following TBI on a systems and behavioral level. Focal injury to the barrel cortex disrupts the pathway’s connectivity and signal transduction, resulting in delayed transneuronal degeneration in areas even outside of the contusion region, such as the thalamus. Therefore, promoting cell survival and functional outcomes requires neuroprotective strategies and re-establishment of the original pathway through endogenous neurogenesis. We delivered a focal injury to the barrel cortex of young adult mice using the well-established controlled cortical impact model of TBI. Wnt3a was administered intranasally once at 1 hr post-injury. At 24 hours post-injury, mice were sacrificed to collect tissue for Western blotting, and at 2 days post-injury, mice were sacrificed for immunohistochemistry. To assess the extent of autophagy, we performed Western blots for Beclin-1 and LC3, two components of the autophagic pathway. Furthermore, we used Beclin-1 and LC3 to immunofluorescently label cells around the peri-contusion region in cryosectioned brains. Our findings suggest that Wnt3a is able to ameliorate cell death by reducing the susceptibility of injured cells to autophagy. These results demonstrate the efficacy of Wnt3a as a treatment during the acute stage of TBI, as well as uncover a novel mechanism of action of Wnt3a that can synergize with its neuroregenerative effects.

Disclosures:  J.Y. Zhang: None. J. Lee: None. S.P. Yu: None. L. Wei: None.

Poster

500. Traumatic Brain Injury: Therapeutic Strategies III

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 500.19/J5

Topic: C.10. Trauma

Support: Medtronic

VA Merit Review B6570R

Title: Acute intrathecal baclofen (ITB) and therapeutic exercise provide effective rehabilitation for TBI-Induced spasticity without adversely affecting cognitive performance

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Abstract: Spasticity is a major health problem for patients with moderate to severe traumatic brain injury (TBI). Current federal guidelines preclude the use of ITB therapy during the first year following TBI due to insufficient data to determine potential risk associated with early therapies on cognitive function, balance, and motor recovery. To address some of these challenges, there were two objectives of the present series of studies. The first was to provide a comprehensive evaluation of dose response using three ITB doses: low (0.4 µg/hr), medium (0.8 µg/hr), and high (1.6 µg/hr) (Lioresal® baclofen injection), initiated at 1 week post injury, and continued for 1 month, on the long-term outcome of spasticity, cognition, function, and balance recovery. Our data to date indicate that compared with time-matched data obtained from untreated TBI animals, ITB treatment significantly reduced spasticity in a dose-dependent manner. The higher dose (1.6 µg/hr) blocked the early (tested at post-ITB treatment week 1) and late onset (tested at post-ITB treatment week 3) spasticity with no negative impact on cognitive performances. However, animals receiving the highest dose, exhibited balance test deficiencies. In contrast, the medium dose blocked the early onset spasticity, significantly attenuated the late onset spasticity, and produced no negative impact on balance and cognitive performances. The lowest dose mildly attenuated early and late onset spasticity with no adverse impact on balance and cognitive performances. The second objective was to use the optimal therapeutic dose in an experimental rehabilitation program that combined ITB and locomotor exercise. In these studies ITB and treadmill locomotor (Tm) exercise were initiated at one week after TBI and continued for 4 more weeks. These studies compared tests of lower limb spasticity, balance, serial learning, and anxiety function in normal, TBI-saline treated, TBI-ITB treated, and TBI-ITB plus Tm animals. One month of ITB treatment using the medium dose (0.8 µg/hr) combined with a Tm locomotor training protocol produced the most significant reduction in spasticity without detectable impact on cognitive, balance, and anxiety recovery. These observations indicated that initiating ITB at one week post-TBI was safe, feasible and effective, and in combination with a Tm locomotor therapeutic exercise training protocol provided the most effective rehabilitation. These studies also indicate that progressively, a broad spectrum of comprehensive data will reinforce confidence in the safety, feasibility, and efficacy of early intervention treatments with locomotor therapy for TBI-spasticity.


Poster

500. Traumatic Brain Injury: Therapeutic Strategies III

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM
Program/#Poster#: 500.20/J6

Topic: C.10. Trauma

Support: Veterans Affairs RR&D Merit Review Grant B6570R

Veterans Affairs RR&D Merit Review Grant B78071

Veterans Affairs RR&D Merit Review Grant B1005-R

Title: Post-traumatic pain in a rodent model of mild-traumatic brain injury (mTBI) and treatment with transcranial magnetic stimulation (TMS)

Authors: *G. MUSTAFA1,2, J. HOU1,2, S. TSUDA2, R. NELSON1, R. M. CAUDLE3, J. K. NEUBERT4, F. J. THOMPSON1,2,5, P. BOSE1,2,6;

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Abstract: Pain is a major complaint in patients after traumatic brain injury and ranges from acute to chronic forms of neuropathic, central, and thalamic pain. It is known that the prevalence of chronic pain is higher (72%) in mild TBI than severe TBI (32%). Although only limited fundamental neurobiological details of TBI-induced pain are available, the causes of pain in mild TBI patients has been attributed to TBI induced neuroplasticity, such as alteration in neurotransmitter signaling in central pain pathways. Accordingly, specific insights into TBI-induced changes in transmitter signaling in pain pathways could direct the development of more effective TBI pain therapies. To obtain a broader understanding of pain in mTBI, we used a rodent model of closed head mTBI (modified Marmarou’s model, 450 g X 1.25 m, anesthetized helmeted adult Sprague Dawley rats). We measured thermal sensitivity in hind-paws and orofacial regions using a conventional hot plate assay and an orofacial pain assessment device respectively before and after mTBI. Our data to date indicate that animals with mild TBI exhibit significant increases in thermal sensitivity starting at 4 weeks post-TBI; this increased sensitivity was enduring and was prominent when the testing was repeated at post-TBI week 8. In parallel with these changes in behavior, significant alterations of key neurotransmitters (eg. 5-HT, GABA, NE and substance P receptors (NKR1)) were found in the sensory cortex, ventral postomedial thalamic nucleus and nucleus caudalis (trigeminal pathway) and also in dorsal root ganglia. Treatment of post TBI animals with a regimen of TMS therapy produced significant decreases in the thermal sensitivity in both facial and peripheral regions. These findings suggest an encouraging potential for TMS in pain management. Accordingly, we propose a working hypothesis that includes TMS as an effective therapy to deal with the alteration of neuromodulators in the pain processing pathway following mTBI.

Poster

500. Traumatic Brain Injury: Therapeutic Strategies III

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 500.21/J7

Topic: C.10. Trauma

Support: Veterans Affairs RR&D Merit Review Grants B6570R,

Veterans Affairs RR&D Merit Review Grants B78071

Veterans Affairs RR&D Merit Review Grants B1005-R

Title: Altered noradrenergic innervation in the amygdala-bed nucleus of the stria terminalis and the ventral subiculum-paraventricular nucleus of hypothalamus anxiety pathways following closed-head traumatic brain injury in rats

Authors: *S. TSUDA*¹, J. HOU¹, R. NELSON¹, G. MUSTAFA¹, Z. WILKIE¹, F. J. THOMPSON¹, P. BOSE¹;

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Abstract: In the United States, each year approximately 1.7 million people experience traumatic brain injuries (TBIs). The majority of these injuries are closed-head TBIs (cTBIs) which often lead to long-term anxiety disorders. Critical questions remain regarding the neurobiology of these disorders, and, accordingly, there has been no established treatment for this morbidity. It is understood that several interconnected brain regions, such as the medial prefrontal cortex (mPFC), amygdala (AMG), the bed nucleus of the stria terminalis (BNST), ventral hippocampus (vHPC), and hypothalamus work in concert to regulate anxiety. Although dysregulation of the central noradrenergic (NA) system has been correlated with anxiety, few studies have specifically reported alteration in the NA innervation (NAI) to known essential mood regulating neural substrates following cTBI. The purpose of this study was to identify chronic TBI-induced changes in the locus coeruleus (LC) neurons (LC supplies NA projections to extensive regions of the central nervous system), TBI-associated changes in the NAI pathways, and NA expression in several mood regulating neural substrate regions. Post-fixed coronal brain sections of animals in which anxiety-like behaviors were detected 5-6 months after cTBI (modified Marmarou TBI
Model, 450g x 1.25 m) were immunofluorescently stained with dopamine-hydroxylase (DH) and norepinephrine (NE). NA fiber density was determined by unbiased stereological analysis using the Petrimetrics method with the Stereoinvstigator software (MicroBrightField). Compared with time-matched normal controls, significantly fewer immunoreactive (IR-) NA cells were detected in the LC. Moreover, significantly fewer IR-NA fibers were detected in the dorsal NA projection pathway as well as the central nucleus of amygdala. In contrast, significantly elevated IR-NA fibers were detected in specific regions of the BNST, subiculum (Sub), and dorsomedial hypothalamic nucleus (DMH). These results suggest that cTBI-induced enduring anxiety disorder may, in part, be related to significant alterations in central NA projections to and within mood regulating neural substrates. Accordingly, these studies provide new information for understanding the anxiety-related cTBI neurobiology, which in turn may provide insights for targeted therapies of cTBI-induced anxiety disorders.


Poster

500. Traumatic Brain Injury: Therapeutic Strategies III

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 500.22/J8

Topic: C.10. Trauma


Title: New therapy in experimental TBI-induced motor (e.g. spasticity and balance), cognitive and anxiety disorders

Authors: *J. HOU*, R. NELSON, Z. WILKIE, G. MUSTafa, S. TSUDA, R. J. BERGERON, Jr., P. BOSE, F. J. THOMPSON; 1NF/SG Veterans Hlth. Syst., Gainesville, FL; 2Dept. of Physiological Sci., 3Dept. of Medicinal Chem., 4Dept. of Neurol., 5Dept. of Neurosci., Univ. of Florida, Gainesville, FL

Abstract: Traumatic brain injury (TBI) can produce life-long disabilities including motor, anxiety and cognitive deficits. Development of safe and effective therapies for these long-term disabilities is urgently needed. Here, we evaluated the therapeutic potential of two treatments
individually: a) transcranial magnetic stimulation (TMS), and b) an iron chelating agent (HBED, mono-sodium salt). We used a rodent model of mild/moderate TBI (modified Marmarou closed head TBI model: 450 g/1.25 m); we recently reported that enduring spasticity, balance, anxiety and cognitive deficits were observed and quantitated in this TBI model (Bose et al., 2013). The TMS treatments were applied 3 times/week for one month and consisted of 75 single pulses delivered to the surface of the cranium through a 25 mm figure of 8 coil using an intensity ladder protocol that we recently reported (Hou et al., 2014). The velocity-dependent ankle torques and time-locked triceps surae EMGs were recorded as a measure of spasticity. Anxiety behavior, balance, and cognitive performance were measured using an elevated plus maze (EPM), rotorod, and Morris water maze (MWM), respectively. Compared to untreated TBI controls, four weeks of TMS treatment resulted in an 87.28% reduction in spasticity, a 25.71% reduction in anxiety score, a 17.67% reduction in balance disability, and a 78.71% increase in serial learning performance. Interestingly, significant therapeutic effects on spasticity reduction (90.05%) and balance improvement (21.14%) persisted for more than 3 months following cessation of TMS treatment. In a different cohort of animals, iron chelator (HBED) treatment (s.q.100 mg/kg/day, twice a day for 10 days, started immediately following the TBI) effectively blocked the development of spasticity and improved the balance performance. These therapeutic benefits were still evident when tested at 4 months following cessation of treatment. No changes were detected in EPM (anxiety) and MWM (learning) performance following chelator treatment. Our data to date indicate that compared with untreated TBI controls, TMS treatments induced significant improvements in cognitive performance and significant reductions in anxiety, balance disability, and spasticity. Chelator treatments blocked the development of motor and balance disabilities. In both treatments, the therapeutic benefit for motor and balance persisted when tested 3 or 4 months after cessation of treatments. Further studies are in progress to understand the role of chronic neuro-inflammation and oxidative stress on these differential therapeutic benefits.


Poster

500. Traumatic Brain Injury: Therapeutic Strategies III

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 500.23/J9

Topic: C.10. Trauma
**Title:** Neurobiological markers of aberrant aversive learning in the traumatic brain injury rodent model

**Authors:** *R. P. MEARS¹, H. C. CROMWELL³, P. K. BOSE⁵, F. J. THOMPSON³;
¹Dept. of Psychology, ²Dept. of Physiological Sci., Univ. of Florida, Gainesville, FL; ³Univ. of Florida, Department of Neurology, FL; ⁴Dept. of Psychology, Bowling Green State Univ., Bowling Green, OH; ⁵Brain Rehabil. Res. Ctr. (151), North Florida/South Georgia VA Hlth. Syst., Gainesville, FL

**Abstract:** Traumatic brain injury (TBI) is accompanied by increased risk of PTSD, persistent anxiety, and dysphoria. Closed head traumatic brain injury (CH-TBI) in the rat model produces emotional dysregulation and anxiety. Diffuse axonal injury in the CH-TBI model accompanies disruption of neuromodulation of prefrontal cortex. Inhibitory gating (IG) deficits coincide with cognitive and emotional dysfunction in a variety of neuropsychiatric disorders. Persistent IG occurs in rat prefrontal medial prefrontal cortex (mPFC), a crucial site for modulating emotional learning. Here we hypothesized that IG would be disrupted in the CH-TBI animal model. Closed head TBI (CH-TBI) rats were assessed in order to identify biological markers of anxiety and disrupted fear conditioning. To investigate the interaction of affect and IG, we recorded local field potentials (LFP) directly from prefrontal mPFC and examined the influence of tone-shock fear conditioning (FC) on IG. In an auditory cue discrimination paradigm, one tone co-terminated with footshock and the other did not. Subjects were tested immediately after and one day after fear conditioning. Behavioral reactions during IG were observed before and after FC, and increase of orienting response after FC indicated induction of tone-shock association. After FC, some components of LFP response exhibited short-term weakening of IG. On a subsequent day of recording, IG strengthened for all LFP components in control animals. These findings will permit further definition and expansion of non-invasive, neuro-diagnostic markers of traumatic brain injury that might significantly improve early detection of TBI associated disorders.

**Disclosures:** R.P. Mears: None. H.C. Cromwell: None. P.K. Bose: None. F.J. Thompson: None.

**Poster**

**501. Cell Death Mechanisms: Oxidative Stress**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 501.01/10

**Topic:** C.11. Neurotoxicity, Inflammation, and Neuroprotection

**Support:** Natural Science Foundation of China 30970664; 31171354
Title: Role of d-serine in nadph oxidase-induced oxidant/antioxidant imbalance in early stage vascular dementia

Authors: *N. LI1, W. ZHANG1, Y. ZHU1,2, D. BRANN2, R. WANG1;
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Abstract: Glutamate excitotoxicity and NADPH oxidase-derived reactive oxygen species (ROS) are important pathological components in neurodegenerative diseases. In the current study, we examined the role of D-serine, a co-agonist at the NMDA class of glutamate receptors, in the early pathogenesis of vascular dementia (VD). Chronic hypoperfusion induced by bilateral common carotid artery occlusion (BCCAO) in the rat was used as the animal model for vascular dementia. The results of our study revealed that a profound oxidant/antioxidant imbalance occurs in hippocampal CA1 neurons at 7d and 21d after BCCAO. For instance, antioxidant factors such as NF-E2-related factor 2 (Nrf2) and its downstream anti-oxidative enzymes (HO-1, SOD2) were significantly decreased after BCCAO, while oxidant factors (O2-) and oxidative stress markers (4-HNE and 3NT) were markedly increased. The elevation of O2- and oxidative stress was likely due to enhanced NADPH oxidase, as in-situ hybridization and activity assays revealed NADPH oxidase expression and activity was significantly enhanced at 1h, 1d, 7d and 21d after BCCAO. The increase of NADPH oxidase appears to involve mediation by D-serine, an NMDA receptor co-agonist, as expression of serine racemase (SR), an enzyme that forms D-serine from L-serine, was significantly increased from 1h to 21d after BCCAO. Furthermore, continuous central administration of antisense oligonucleotides (AS) of SR not only markedly attenuated the activity of NADPH oxidase, 4HNE and 3NT, but also up-regulated the protein expression of the anti-oxidant enzymes Nrf2, HO-, and SOD2, as compared to the BCCAO 21d group. Of significant interest, both SR knockdown and gp91ds-tat, a specific inhibitor of NADPH oxidase, perfectly prevented early ultrastructural damage in hippocampal CA1 neurons at 21d after BCCAO. Taken as a whole, the results suggest that up-regulation of endogenous D-serine in the hippocampal CA1 region plays an important role in the NADPH oxidase induction and antioxidant/oxidant imbalance that occurs during early stage BCCAO.


Poster

501. Cell Death Mechanisms: Oxidative Stress

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 501.02/J11
Role of oxidative cysteine protein modification in Amphetamine induced neurotoxicity

Authors: V. BHARTI, H. TAN, Z. ZHOU, Y. WANG, *J.-F. WANG;
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Abstract: Amphetamine is a highly abused psycho stimulant that can lead to dopaminergic neuron degeneration and increased the risk for neurodegenerative diseases with advanced age upon long term consumption. Many studies have shown that oxidative/nitrosative stress contributes significantly to AMPH-induced toxicity. Thiols of cysteine residues in many proteins are very susceptible to attack by reactive oxygen/nitrogen species H2O2 and NO$, subsequently inducing sulfenylation and nitrosylation. In this study, we analyzed the effects of amphetamine on H2O2 induced sulfenylation and NO$ induced nitrosylation in rat brain using biotin switch method following by immunoblotting analysis. We found that repeated amphetamine treatment increased total sulfenylation and nitrosylation of proteins in rat frontal cortex. Vesicular monoamine transporter 2 (VMAT2) is mainly responsible for packaging of monoamine neurotransmitters and play an important role in neurotransmission. We found that VMAT2 can be sulfenylated and nitrosylated, and that repeated amphetamine treatment increased sulfenylation of VMAT2. Our findings suggested that nitrosylation and sulfenylation of proteins including VMAT2 may interrupt normal regulation of neurotransmission and lead to neurotoxicity


Poster

501. Cell Death Mechanisms: Oxidative Stress

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 501.03/J12

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: KAKENHI 15H04999
KAKENHI 26861479
KAKENHI 25430082
Title: Neuroprotection by valproic acid and spermidine in a mouse model of normal tension glaucoma


Abstract: Glaucoma is characterized by progressive degeneration of retinal ganglion cells (RGCs) and their axons, together with visual field loss, which are usually associated with elevated intraocular pressure (IOP). Normal tension glaucoma (NTG) is a subtype of glaucoma that presents with statistically normal IOP. We previously reported that loss of glutamate transporters (EAAC1 or GLAST) in mice leads to RGC degeneration that is similar to NTG (J Clin Invest 117:1763-70, 2007). These mice show elevated glutamate neurotoxicity and oxidative stress, which have been proposed to contribute to human glaucoma. Valproic acid (VPA) is widely prescribed for treatment of epilepsy, mood disorders and migraines. It modulates multiple mechanisms including glutamate neurotransmissions, activation of pro-survival protein kinases and inhibition of histone deacetylase. Herein, we show that VPA (300 mg/kg) treatment prevented RGC death and visual disturbance in GLAST knockout (KO) mice without affecting IOP. We found that VPA reduces oxidative stress induced in the GLAST KO retina and stimulates the cell survival signalling pathway associated with extracellular-signal-regulated kinases (ERK). Polyamines, such as spermidine, are organic cations required for cell growth, cell differentiation, and synthesis of DNA, RNA, and proteins. Spermidine plays key roles in mediating protection against oxidative damage. We show that spermidine, at 30 mM in drinking water, prevented retinal degeneration and improved visual function in EAAC1 KO mice without affecting IOP. Spermidine alleviated the severity of the glaucoma-like phenotype by acting as an antioxidant. Our findings raise intriguing possibilities that the widely prescribed drug VPA and oral spermidine administration could be useful for treating retinal degenerative disorders including glaucoma. In addition, our NTG models are available to test the effect of a variety of therapeutic interventions including novel and existing drugs, particular foods, and gene therapy, in a short period.

**Abstract:** Iron is essential for proper neuronal functioning; however, excessive accumulation of brain iron is reported in Parkinson’s, Alzheimer’s, Huntington’s diseases and amyotrophic lateral sclerosis. This indicates that dysregulated iron homeostasis is involved in the pathogenesis of these diseases. In this work, we examined the molecular mechanisms activated by iron in neuroblastoma SH-SY5Y cells. We found that iron induced apoptotic cell death in SH-SY5Y cells in a concentration-dependent manner. Detection of iNOS and nitrotyrosine confirms the presence of increased reactive oxygen and nitrogen species. Furthermore, we found a decrease of catalase and protein arginine methyl-transferase 1 (PMRT1). Interestingly, iron increased the activity of ERK and AKT and reduced DyrK1B. Moreover, after FeCl2 treatment, the transcription factors c-Jun and pSmad1/5 were activated. These results indicate that the presence of high levels of iron increase the vulnerability of neurons to oxidative stress.

**Disclosures:** E. Bautista: None. P. Vergara: None. J. Segovia-Vila: None.
Title: Acute binge ethanol exposure induces brain proinflammatory cytokines, NADPH oxidase, microglial activation and neurodegeneration

Authors: *L. QIN, F. T. CREWS;
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Abstract: Binge drinking levels of alcohol increase endotoxin and innate immune signaling molecules in blood. In previous studies we found that 10 days of ethanol treatment of mice activated blood and brain innate immune gene induction including proinflammatory cytokines and NADPH oxidase (NOX) that forms reactive oxygen species (ROS) that lead to neurodegeneration. We investigated acute binge alcohol drinking responses in brain using male C57BL/6J mice treated with 1 oral binge dose of ethanol (6 g/kg, i.g., 25% ethanol w/v). We determined mRNA and protein levels of TNFα, IL-1β and MCP1 as well as NOX and ROS using real-time PCR, immunohistochemistry and hydroethidine histochemistry. Acute binge ethanol treatment increased activated caspase-3 +IR cells in the entorhinal cortex and reduced neurogenesis (i.e. doublecortin immunoreactivity) in the dentate gyrus 24 hours after ethanol treatment, consistent with increased cell death. Iba1 +IR indicated activated morphology of microglia with increased NOX gp91phox expression 24 hours after ethanol exposure that remained elevated for at least 1 week after exposure. Ethanol increased gp91phox expression coincided with increased production of O2- and O2--derived oxidants. Diphenyleneiodonium (DPI), a NOX inhibitor, reduced brain gp91phox gene expression, release of ROS and microglial activation. Acute ethanol increased HMGB1 and TLR4 immunoreactivity 24 hours after ethanol treatment. Inhibition of HMGB1 by Glycyrrhizin, a HMGB1 inhibitor, decreased ethanol-induced gp91phox and ROS, as well as microglial activation. These results suggest that HMGB1 is associated with up-regulation of NOX and release of ROS after acute ethanol treatment. Further, acute ethanol induction of NOX and production of ROS may contribute to “hangover-like” acute ethanol withdrawal responses as well as long lasting microglial activation and neurodegeneration.

Disclosures: L. Qin: None. F.T. Crews: None.

Poster

501. Cell Death Mechanisms: Oxidative Stress

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 501.06/J15

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: MDA Grant 294842
Title: Novel function of amyotrophic lateral sclerosis-associated fus in oxidative DNA damage repair by enhancing the ligation activity of ligase III

Authors: *H. Wang¹, T. Alan E.², P. Hegde¹, S. Mitra³, M. Hegde⁴; ¹Houston Methodist Hosp. Res. Inst., Houston, TX; ²Univ. of New Mexico Cancer Center, University of New Mexico, Albuquerque, NM; ³Houston Methodist Res. Inst., Houston, TX; ⁴Houston Methodist Res. Inst., Houston, TX

Abstract: The RNA/DNA-binding protein named Fused in Sarcoma/Translocated in Sarcoma (FUS/TLS) has been etiologically linked to the initiation and progression of motor neuron degenerative disorder, amyotrophic lateral sclerosis (ALS). While the molecular basis for many sporadic forms of ALS is unknown, the others result from hereditary mutations in the FUS/TLS gene that leads to cytoplasmic aggregation of the polypeptide. Recent studies, showing ATM-mediated phosphorylation of FUS and the latter’s interaction with DNA single-strand break marker protein PARP1, suggested its role in DNA damage response. However, direct involvement of FUS in DNA repair and its pathological implications in motor neuron death in ALS is still unexplored. Furthermore, significantly higher DNA damage observed in the motor cortex and other affected brain regions of familial ALS patients harboring FUS mutations than in the control, warrants investigation of the role of FUS in DNA damage repair. Here we have observed that FUS’s specific in-cell complex with XRCC1 and Ligase III but not with XRCC4 and Ligase IV, the association increased after oxidative stress in SH-SY5Y cells as well as in human motor neural cells derived from neural stem cells (hNSC line). In vitro co-elution analysis revealed that FUS physically interacts with both XRCC1 and Ligase III, which significantly stimulates SSBR efficiency by enhancing the catalytic activity of ligase III, an essential enzyme that ligates DNA nicks. Furthermore, shRNA-mediated FUS knock-down sensitized neurons to oxidative stress. Taken together, our data suggest that FUS is required for oxidative DNA damage repair via its functional regulation of DNA ligase III activity. Together with nuclear clearance of FUS in both sporadic and familial ALS, our data provides clues of how FUS mutation negatively affects oxidative damage repair in the genome and thus could open up new avenues for therapeutic intervention in ALS. (Hegde laboratory is supported by Muscular Dystrophy Association and ALS Association and NIH grant R01 NS088645).


Poster

501. Cell Death Mechanisms: Oxidative Stress
Title: Sirt3-mediated deacetylation of SOD2 contributes to neuronal survival during excitotoxicity

Authors: *C. C. ALANO, S. KIM; NEUROLOGY, VAMC/UCSF, San Francisco, CA

Abstract: Sirt3 is a major NAD-dependent deacetylase in mitochondria, and has been shown to regulate energy metabolism and oxidative stress response. In our previous study, we showed that Sirt3 plays a direct role in protecting neurons from NMDA-induced excitotoxicity. However, the mechanism by which Sirt3 mediates neuroprotection in mitochondria during excitotoxicity is not clear. Here, we show that Sirt3 deacetylates SOD2, which reduces mitochondrial ROS production upon NMDA-induced excitotoxicity. An increase in Sirt3 correlated with SOD2 deacetylation during NMDA-induced excitotoxicity. Acetylated SOD2 was not reduced in Sirt3-KO neurons under excitotoxic condition, whereas SOD2 was more deacetylated in NMDA-treated WT neurons. Neurons from Sirt3-KO had a larger increase in reactive oxygen species (ROS) production than the wt neurons after exposure to excitotoxic insult. Neurons from Sirt3 KO mice showed a decrease in mitochondrial respiration and ATP production compared to wt neurons under either control or excitotoxic conditions. NMDA-induced neuronal death was higher in neurons from Sirt3 KO mice than WT mice, which was attenuated by SOD2 mimetics. Our study demonstrates that Sirt3-mediated deacetylation of SOD2 contributes to neuroprotection from excitotoxicity.

Disclosures: C.C. Alano: None. S. Kim: None.
Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: UK Medical Research Council

Wellcome Trust

Biotechnology and Biological Sciences Research Council

Royal Society

'Nplast' Marie-Curie Initial Training Network

Fidelity and a Biogen Idec/University of Edinburgh Joint Discovery Research Collaboration

Title: Neuronal activity and the astrocytic Nrf2 pathway cooperate to provide neuroprotection against oxidative stress

Authors: *N. M. MARKUS*¹, K. F. S. BELL¹, P. S. BAXTER¹, B. AL-MUBARAK¹, M.-A. MARTEL¹, N. WHEELAN¹, S. MCKAY¹, R. F. DEIGHTON¹, P. HASEL¹, S. CHOWDHRY², P. J. MEAKIN², A. M. KAINDL³, R. H. SCANNEVIN⁴, D. J. A. WYLLIE¹, J. D. HAYES³, G. E. HARDINGHAM¹;

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Abstract: Nuclear factor erythroid 2-related factor 2 (Nrf2) is a master regulator of antioxidant genes, and its activation in astrocytes is known to provide non cell-autonomous neuroprotection against oxidative insults. However, the capacity for dynamic regulation of Nrf2 target genes in neurons, and their role in boosting intrinsic antioxidant defences, is poorly understood. We examined the contributions of Nrf2 target genes in neurons and astrocytes in providing neuroprotection. We found that, in contrast to astrocytes, the Nrf2 pathway is inactive in neurons due to the Nrf2 promoter being epigenetically silenced during early development. However, this did not mean that Nrf2 target gene expression could not be controlled. Known Nrf2 target genes, including genes involved in the glutathione antioxidant system, were induced in neurons following neuronal activity. However, this gene induction was found to be independent of Nrf2, being observed in Nrf2-deficient neurons. Since neuronal activity is associated with higher metabolic activity and ROS production, and higher glutathione usage, this gene induction serves to tune the neuron's defences to reflect higher requirements of an active cell. Of note, we found that neuronal activity and astrocytic Nrf2 activation were additive in promoting neuroprotection against oxidative insults and sustaining neuronal glutathione levels, demonstrating the importance of both autonomous and non-cell autonomous pathways. To conclude, despite having a non-functional Nrf2 pathway, neuronal activity can promote the expression of many known antioxidant Nrf2 target genes to modify intrinsic antioxidant defences.

**Poster**

**501. Cell Death Mechanisms: Oxidative Stress**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 501.09/J18

**Topic:** C.11. Neurotoxicity, Inflammation, and Neuroprotection

**Title:** TGF-β1 upregulates system xc- through ERK mediated increased oxidative stress

**Authors:** *R. ALBANO, J. HJELMHAUG, D. LOBNER;
Marquette Univ., Milwaukee, WI

**Abstract:** System xc-, the cystine/glutamate exchanger located on the cell membrane, mediates the transport of one cystine molecule into the cell in exchange for the release of one glutamate molecule into the extra extrasynaptic space. Through providing cystine to the cell system xc- regulates the levels of cellular glutathione (GSH), the main endogenous intracellular antioxidant, and in this way may protect cells against oxidative stress. However, by releasing glutamate, it can increase extracellular glutamate levels and potentially cause excitotoxicity. Due to this dual nature, system xc- likely plays an important role in regulating neuronal survival and death. In order to better understand the role system xc- plays in neuronal survival and death how it is regulated must be understood. We have found that transforming growth factor β1 (TGF-β1), which is involved in many cellular processes, increases cystine uptake through system xc- in both a concentration and time-dependent manner in murine primary cortical cultures, with the effect being specific to astrocytes. TGF-β1 treatment also induced an early increase in oxidative stress (1 and 3 hours). The ERK antagonist, U0126, blocked both the early increase in oxidative stress and the upregulation of system xc-, suggesting that both are downstream of ERK activation. The antioxidant allopurinol also decreased the TGF-β1 induced increase in system xc-. Together the data suggest that the upregulation of system xc- by TGF-β1 occurs through an early ERK-dependent increase in oxidative stress. Since system xc- regulates the amount of GSH in cells and the TGF-β1 induced increase in oxidative stress was back to control levels by 24 hours, we hypothesized that the upregulation of system xc- by oxidative stress increased intracellular GSH levels. Interestingly, we found that TGF-β1 did not change GSH in mixed (neural and glial) cultures. However, it decreased levels of GSH in pure glial cultures, but increased the amount of
GSH in the media. In pure neuronal cultures TGF-β1 decreased GSH levels both in the cells and in the media. We hypothesized that TGF-β1 was causing glial cells to export GSH, so that it can be taken up by and used to protect neurons. To test this we used a cell culture insert system that allowed us to co-culture glial cells and pure neurons together, but then separate them to assess GSH levels in each of the cell types separately. In this system TGF-β1 still decreased GSH levels in glial cells, however, GSH levels were maintained in the neurons. This data supports our hypothesis that glial cells are exporting GSH, which is being taken up by neurons, even to the detriment of the glial cell antioxidant defenses.

**Disclosures:** R. Albano: None. J. Hjelmhaug: None. D. Lobner: None.

**Poster**

**501. Cell Death Mechanisms: Oxidative Stress**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 501.10/J19

**Topic:** C.11. Neurotoxicity, Inflammation, and Neuroprotection

**Support:** Grant ICYTDF/229/2012

**Title:** Effect post-lesion of curcumin on quinolinic acid-induced dysregulation of MAPK pathway signaling in a model of striatal neurodegeneration

**Authors:** *R. A. SANTANA MARTINEZ¹, D. BARRERA², P. D. MALDONADO³;
¹Natl. Inst. of Neurol. and Neurosurg., Mexico, Mexico; ²Univ. Nacional Autónoma de México, Mexico City, Mexico

**Abstract:** Quinolinic acid (QUIN), an endogenous competitive agonist of NMDAr, is considered an excitotoxin and its intrastriatal administration to rats has been used to reproduce some biochemical, behavioral and morphological alterations similar to those observed in some neurodegenerative disorders. QUIN induces selective neuronal death in the striatum due to overactivation of NMDAr (excitotoxicity), which has been related to acute oxidative stress. It has been checked that the oxidative stress-induced MAPK signaling pathway activation is often dysregulated in pathological conditions. Oxidative stress activates in some cases MAPK signaling to further the survival cell, but also the activation of the same pathway, promotes cell death. Emerging evidence suggest that some naturally occurring antioxidants exert their health-promoting effects by activating one or more adaptive cellular stress response pathways. Curcumin (CUR), a polyphenol derived from the rhizome of Curcuma longa is highly lipophilic and its biological properties as direct (free radical scavenger) and indirect (Nrf2 inducer)
antioxidant are well known. In this study, we evaluate the role of CUR on activation or inactivation on MAPK kinases pathway induced by QUIN and its protective effect on QUIN-induced morphological and behavioral alterations. Animals were intrastriatally infused with QUIN (30, 60, 120 and 240 nmol/µl) and after 24 h, received CUR (400 mg/kg, i.g.) daily during 6 consecutive days. CUR decreased the histological damage and number of neurons positive to FJ-B (7 days post-lesion) as well as QUIN-induced behavioral alterations in doses-dependent manner. We found that QUIN (120 and 240 nmol/µl) significantly decreased the level of phospho-ERK1/2 respect to control at 7 day. Stress-activated signaling pathway JNK and p38 were activated by QUIN at highest doses. The impairment in phospho-ERK1/2 could be related to decrease in BDNF (brain-derived neurotrophic factor) and TrkB (BDNF-receptor) levels, at same doses of QUIN. So far, our results suggest that QUIN turn off the survival-related signaling pathways and turn on death-related signaling pathways, possibly by oxidative stress. Further studies are needed to elucidate the effect of CUR and precise its mechanism action to explain the toxic paradigm induced by QUIN on MAPK signaling pathways.


Poster

501. Cell Death Mechanisms: Oxidative Stress

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 501.11/20

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant RO1 NS27073

Title: The impact of reactive oxygen species (ROS) on mitochondrial transport in axons

Authors: *P.-C. LIAO, P. J. HOLLENBECK; Biol. Sci., Purdue Univ., West Lafayette, IN

Abstract: Mitochondria are the source of aerobic ATP production for the cell and also contribute to calcium (Ca²⁺) homeostasis. To maintain cellular function and survival in neurons, mitochondria are transported along the axon between different domains, and accumulate in regions with high demand for their functions. But mitochondria are also a major source of reactive oxygen species (ROS) production, which can induce oxidative damage. Both oxidative stress and abnormal mitochondrial axonal transport induce neurodegenerative disorders. However, we know little about the connection between oxidative stress and mitochondrial transport. Here we used primary Drosophila neuronal cell culture and the third instar larval
nervous system as *in vitro* and *in vivo* models, respectively, to study mitochondrial transport under oxidative stress conditions. In primary neuronal cell culture, hydrogen peroxide treatment resulted in reduced mitochondrial length and diminished membrane potential, judged by ratiometric imaging with tetramethylrhodamine methyl ester (TMRM). Both *in vitro* and *in vivo*, we found that ROS treatment inhibited several parameters of mitochondrial transport including flux, percentage of moving mitochondria, velocity, duty cycle, and run length. Furthermore, these defects were rescued by the overexpression of superoxide dismutase 1 (SOD1). Our findings indicate that oxidative stress not only affects mitochondrial morphology and membrane potential, but also reduces their axonal transport both *in vitro* and *in vivo*. To further understand the mechanisms behind these results, we activated the JNK pathway, which has been shown both to respond to oxidative stress and to inhibit fast axonal transport. JNK activation yielded reduced mitochondrial flux and velocities in both directions. In addition, JNK knockdown partially rescued defects in mitochondrial transport under oxidative stress conditions, suggesting that the JNK pathway plays a role in regulation of mitochondrial transport in response to ROS. In addition, we found higher Ca²⁺ levels, which has been shown to abolish mitochondrial transport, under oxidative stress conditions by using genetically encoded Ca²⁺ indicator (GCaMP6). These results suggest that in addition to the JNK pathway, Ca²⁺ levels are involved in regulation of mitochondrial transport in the presence of ROS.

**Disclosures:** P. Liao: None. P.J. Hollenbeck: None.

**Poster**

**501. Cell Death Mechanisms: Oxidative Stress**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 501.12/J21

**Topic:** C.11. Neurotoxicity, Inflammation, and Neuroprotection

**Title:** Inhibition of HDAC6 protects neuronal cells against oxidative stress-induced cell death

**Authors:** *L. MA, Y. HU, C. A. GRETZULA, S. NIROOMAND, J. J. RENGER, S. M. SMITH; Dept. of Neurosci., Merck Res. Labs., West Point, PA

**Abstract:** Histone deacetylase 6 (HDAC6) plays a central role in α-tubulin acetylation, axonal transport, and neuronal oxidative stress. Inhibition of HDAC6 shows neuroprotective effects by increasing the acetylation of α-tubulin, resulting in a subsequent improvement of axonal transport of mitochondria and reversing axonal loss. Genetic deletion and inhibition of HDAC6 also delays disease progression in symptomatic deficits in neurodegenerative disorders and
motor neuron diseases. Furthermore, HDAC6 is involved in deacetylation of antioxidant enzymes peroxiredoxin-1 (Prx-1) and peroxiredoxin-2 (Prx-2), both of which primarily function to reduce hydrogen peroxide levels in the cell. Evidence shows that HDAC6 inhibition reduces oxidative stress through acetylation of the peroxiredoxins. Therefore, genetic and pharmacological inhibition of HDAC6 has the potential to be a novel therapeutic for the treatment of neurodegenerative disorders associated with oxidative damage. Here we report that the HDAC6 inhibitor Tubastatin A protects rat B35 neuroblastoma cells from L-homocysteic acid (HCA) and hydrogen peroxide induced oxidative stress in a dose dependent manner. This protective effect is also observed with oxidative stress induced apoptotic cell death. Furthermore, Tubastatin A protects primary hippocampal neurons from hydrogen peroxide induced oxidative stress demonstrating efficacy in a native system. Further study reveals that one possible mechanism for Tubastatin A’s neuroprotective properties is its ability to significantly decrease peroxiredoxin superoxidation as demonstrated in hydrogen peroxide treated B35 cells. Since acetylation of Prx increases reductive properties and resistance to superoxidation, HDAC6 inhibition enhances the antioxidant properties of B35 cells by preventing deacetylation of Prx under extreme oxidative stress conditions. Taken together, inhibition of HDAC6 is a very promising novel therapeutic approach to treating oxidative stress associated neurodegenerative diseases.

Disclosures: L. Ma: A. Employment/Salary (full or part-time); Merck & Co. Inc. Y. Hu: A. Employment/Salary (full or part-time); Merck & Co. Inc. C.A. Gretzula: A. Employment/Salary (full or part-time); Merck & Co., Inc. S. Niroomand: A. Employment/Salary (full or part-time); Merck & Co., Inc. J.J. Renger: A. Employment/Salary (full or part-time); Merck & Co., Inc. S.M. Smith: A. Employment/Salary (full or part-time); Merck &Co., Inc.

Poster

501. Cell Death Mechanisms: Oxidative Stress

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 501.13/J22

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: Faculty of Graduate Studies, Mahidol University

Title: Inorganic mercury-mediated cytotoxicity and oxidative injury in human astrocytes
Authors: *D. OSPONDPANT*¹, P. CHIVICHIT¹, N. SIBMOOH¹, S. SOODVILAI², P. VIVITHANAPORN³;
¹Pharmacol., ²Physiol., Fac. of Science, Mahidol Univ., Bangkok, Thailand

**Abstract:** Mercury is a highly neurotoxic heavy metal, leading to brain injury and neurological disorders including Alzheimer's disease. However, its toxic effects on human astrocytes, major glial cells, remain unclear. In the central nervous system, methyl mercury, a common form of organic mercury, can be demethylated and became inorganic divalent mercury, a mercuric form. This study, we aimed to compare the toxic effects of inorganic mercury, mercuric chloride, on human astrocyte cells with human neuronal cells and investigate the mechanism underlying its toxicities. Cell viability was evaluated by MTT reduction assays. Surprisingly, our finding demonstrated that mercuric chloride was toxic to human astrocytoma U373, U-87 MG and A172 cells at concentrations similar to the toxic concentration on human cortical neuronal HCN-2 cells. At 6 hours post-exposure, the median toxic concentration (TC50) values were ranged from 10.1-13.9 µM in human astrocytes and 14.3 µM in human neurons. We further studied the effects of mercuric chloride on reactive oxygen species (ROS) levels and lipid peroxidation production in human astrocytes using dichlorofluorescein (DCF) and thiobarbituric acid reactive substances (TBARs) assays, respectively. Our finding showed that mercuric chloride, 10-100 µM, increased DCF fluorescence intensity at 1 hour post-exposure and malonaldehyde levels at 24 hours post-exposure, indicating that inorganic mercury promote ROS and lipid peroxidation formation. Taken together, these data suggested that inorganic mercury-induced toxicity in human astrocytes was associated with oxidative stress. The toxicities of inorganic mercury to human astrocytes could contribute to pathophysiology of mercury-mediated neurodegeneration.

**Disclosures:** D. Ospondpant: None. P. Chivichit: None. N. Sibmooh: None. S. Soodvilai: None. P. Vivithanaporn: None.

**Poster**

501. **Cell Death Mechanisms: Oxidative Stress**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 501.14/J23

**Topic:** C.11. Neurotoxicity, Inflammation, and Neuroprotection

**Support:** National Natural Science Foundation of China 81125010, 81030025

The Ministry of Science and Technology of China 973-2012CB910701, 2013DFA31990

**Title:** Hippo/MST1 signaling regulates neuronal cell death and microglial activation
Authors: *Z. YUAN, S. ZHAO, L. ZHOU, R. WU;
Inst. of Biophysics, Beijing, China

Abstract: Oxidative stress influences neuronal cell survival and homeostasis, but the mechanisms underlying the biological effects of oxidative stress remain to be elucidated. We have shown that protein kinase Hippo/MST1 plays a major role in oxidative stress-induced cell death in primary mammalian neurons and the protein kinase c-Abl phosphorylates MST1 at Y433, which triggers the stabilization and activation of MST1, thereby activating the MST1-FOXO signaling pathway, leading to cell death in both primary culture neurons and rat hippocampal neurons. Microglial activation has been implicated as a secondary and detrimental cellular response for neuronal cell death in neurodegenerative diseases. Recently we found that MST1 is also involved in the microglial activation through directly phosphorylating IκBα to initiate the immune response. We further identified that Src kinase functions as the upstream of MST1-IκBα signaling during microglial activation. Taken together, we demonstrated that Hippo/MST signaling plays a critical role in both neuronal cell death and microglial activation upon oxidative stress, with the implication of a therapeutic target for the neurodegenerative diseases.

Disclosures: Z. Yuan: None. S. Zhao: None. L. Zhou: None. R. Wu: None.
number of complex adult-onset disorders like cardiovascular diseases, diabetes, neurological disturbances, etc. We hypothesized that increased oxidative stress and reduced activity of antioxidant enzymes along with DNA damage are associated with the etiology of these complications. As little is known about the effects of maternal vitamin B12 restriction on brain regions of the offspring, we have focused particularly on cerebral cortex and hippocampus in our study. Female, weaning C57BL/6 mice received ad libitum for 4 weeks a control diet (American Institute of Nutrition-76A) or the same with restriction of vitamin B12. After confirming the deficiency, the mice were allowed to breed with control males to obtain the F1 generation offspring. The different parameters related to oxidative stress and DNA damage were assessed at 3 months age of the offspring. Interestingly, offspring born to mice fed on vitamin B12 restricted diet had significantly higher degree of oxidative stress in both cerebral cortex and hippocampus as reflected by their increased levels of lipid peroxidation and protein oxidation. Also, the activity of antioxidant enzymes (superoxide dismutase and catalase) was diminished in both the brain regions of offspring born to mice fed on vitamin B12 restricted diet. DNA damage in the form of single and double stranded DNA breaks were also increased in the cerebral cortex and hippocampus of mice offspring born to vitamin B12 restricted dams. To conclude, maternal vitamin B12 deficiency is associated with increased oxidative stress in the brain of young offspring, which further leads to DNA damage in brain.


Poster

501. Cell Death Mechanisms: Oxidative Stress

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 501.16/J25

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH R01NS078026

NIH R01AT007317

AHA Mid-Atlantic Affiliate Grant-in-Aid 13GRNT15730001

AHA Mid-Atlantic Affiliate Postdoctoral Fellowship Award 14POST20140003

Title: Flavanoid (-)-epicatechin inhibits hemoglobin-induced oxidative stress in astrocytes via nrf2 and ap1 pathways
**Authors:** *X. LAN, J. WANG;*  
Johns Hopkins, Baltimore, MD

**Abstract:** (-)-Epicatechin (EC) is a natural flavonoid extracted from cocoa and green tea. Our previous studies have shown that EC improves early intracerebral hemorrhage (ICH) outcomes by modulating redox oxidative stress via the NF-E2-related factor (Nrf) 2 pathway. In this study, we cultured astrocytes from wild-type (WT) and Nrf2 null (Nrf2−/−) mice to explore the effects and molecular mechanisms of EC on reactive oxygen species (ROS) production. In astrocytes from WT mice, EC (100 µM) significantly increased Nrf2 translocation after hemoglobin stimulation and upregulated its downstream target protein expression, including SOD1 and NQO1. However, (-)-epicatechin tended to decrease heme oxygenase 1 (HO1) expression in WT astrocytes exposed to hemoglobin. In Nrf2−/− astrocytes exposed to hemoglobin, (-)-epicatechin did not affect SOD1 or NQO1 protein expression, but it reduced HO1 protein expression. Furthermore, it protected WT and Nrf2−/− astrocytes, increased their viability after hemoglobin stimulation, and decreased ROS production in a dose-dependent manner. The IC50 of EC for inhibition of ROS production was 52.5 µM and 44.1 µM, in WT and Nrf2−/− astrocytes, respectively. These data indicate that the effect of EC on ROS production is not totally Nrf2-dependent. Moreover, we found that in Nrf2−/− astrocytes, EC inhibited translocation of activator protein-1 (AP1 [c-jun and c-fos]), which might control the transcription of HO1. Together, these data suggest that EC inhibits hemoglobin-induced ROS production in astrocytes via Nrf2 and AP1 pathways and that AP1 could be a new molecular target for treating ICH. **Keywords:** (-)-Epicatechin, redox oxidative stress, NF-E2-related factor 2, activator protein-1

**Disclosures:** X. Lan: None. J. Wang: None.

**Poster**

**501. Cell Death Mechanisms: Oxidative Stress**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 501.17/J26

**Topic:** C.11. Neurotoxicity, Inflammation, and Neuroprotection

**Support:** CONACyT 241655

**Title:** Characterization of the Nrf2 activation pathway, independent of oxidative stress

**Authors:** *C. A. SILVA¹, P. MALDONADO²;
²Vascular Cerebral Patology, ¹Natl. Inst. of Neurol. and Neurosurg., Mexico City, Mexico
Abstract: Excitotoxicity is an event that occurs in neurodegenerative disorders involving overactivation of NMDAr, which increase the intracellular calcium and generate an acute state of oxidative stress. Quinolinic acid (QUIN) is a selective agonist of NMDAr capable of inducing an oxidative/nitrosative state in the cell, so its intrastriatal administration has been used as an excitotoxic/pro-oxidant model to study both events. The cells counteract the oxidative stress by activation of Nrf2 factor, a master regulator of the cellular antioxidant responses. The canonical pathway of Nrf2 activation involves the oxidation of the some Keap1 cysteins (protein that retains Nrf2 in the cytoplasm), subsequent dissociation of the complex Keap1-Nrf2 and the Nrf2 translocation to the nucleus. However, a not canonical pathway has been reported in vitro recently. This pathway involves the disruption of Keap1-Nrf2 complex by direct interaction of some proteins with Keap1 or Nrf2, so in this work we propose to evaluate the effect of different doses of QUIN in an in vivo model, on Nrf2 factor activation and these proteins, and its relationship with the state of oxidative stress in the rat striatum. We administrated 1 µL of isotonic saline or QUIN (15, 30, 60, 120 and 240 nmol) in the right striatum of male Wistar rats (260-300 g) and then we sacrificed the animals at 30 min after injection. The Nrf2 activation was measured using an ELISA kit in nuclear extract, the oxidative stress level was evaluated by GSH/GSSG levels and the lipid oxidation by TBARS assay, and finally we evaluated the enzymatic activity of GR, GST, GPx, GCL, G6PDH and CAT like a cellular redox state. The activation of Nrf2 increased 30 min after the QUIN administration in a dose-response manner. The lipid oxidation and the levels of GSH/GSSG showed not changes, but the administration of 120 and 240 nmol of QUIN slightly increased the levels of GSSG. Finally, the enzymatic activity showed no changes with all doses of QUIN. These results suggest that at 30 min, the oxidative stress is not involved in the activation of Nrf2 induced by QUIN administration.

Disclosures: C.A. Silva: None. P. Maldonado: None.

Poster

501. Cell Death Mechanisms: Oxidative Stress

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 501.18/J27

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Title: The ketone body b-hydroxybutyrate (BHB) reduces the production of reactive oxygen species and prevents neuronal death induced by glucose deprivation in cortical cultured neurons
Abstract: Glucose is the most important energy source in brain and whenever its concentration in blood decreases to less than 20 mg/dl, neuronal death can take place. The mechanisms leading to neuronal death during glucose deprivation have not been completely elucidated but the role of reactive oxygen species (ROS) has been suggested. Under certain conditions the brain can consume alternative substrates to glucose, such as the ketone bodies (KB), beta-hydroxybutyrate (BHB) and acetoacetate (AcAc). KB blood levels substantially increase (from 0.1 mM to 1-8 mM) during the ketogenic diet, prolonged fasting and sustained hypoglycemia. It has been shown that the ketogenic diet reduces the number of seizures in epileptic patients and BHB administration prevents neuronal death induced in models of brain ischemia, trauma and neurodegenerative diseases. We have investigated if BHB can prevent neuronal death induced by glucose deprivation (GD) in cortical neurons and whether this effect is related to the decrease in reactive oxygen species (ROS). Primary cortical cultures obtained from E17 embryos were exposed at 8 DIV to GD during 1-2 h to GD followed by glucose reintroduction (GR). ROS production was assessed by the oxidation of the fluorescent dye, dihydroethidium (DHE), ATP levels measured by the luciferin-luciferase determination kit and cell viability assessed by the MTT reduction and the LDH release assays. Results show that D-BHB reduces 50-60% ROS generated during GD and GR, partially prevents the decline in ATP levels and reduces 50-70% neuronal death. D-BHB was effective when administered either only during GD or only during GR. The non-physiological isomer of BHB, L-BHB was less efficient preventing neuronal death as compared with D-BHB, but also effectively reduced ROS generated during GR. Results suggest that protection by BHB, results from its metabolic action combined with its capability to reduce ROS levels, probably due to its contribution to the maintenance of mitochondrial activity. They also suggest that BHB may be a good candidate for the treatment of ischemic and traumatic injury. This work was supported by IN204213 PAPIIT (UNAM) grants to LM.
Support: Purdue University

Title: Novel acrolein scavenger dimercaprol mitigates acrolein-mediated PC-12 cell death, reduces acrolein concentration and offers neuroprotection in a rat contusive spinal cord injury model

Authors: *R. TIAN¹, R. SHI²;

Abstract: Acrolein is one of the most toxic byproducts of lipid peroxidation with a highly electrophilic chemical structure. Acrolein can react with many nucleophilic biomacromolecules including proteins, lipids and DNA, resulting in their dysfunction. As both the product and catalyst of lipid peroxidation, acrolein perpetuates oxidative stress (OS) and OS-related damages in spinal cord injury (SCI). As such, acrolein is believed to play a critical role in neuronal degeneration, particularly the expansion and worsening pathology during secondary injury, which ultimately leads to functional loss after SCI. Therefore, inhibition of secondary injury processes, particularly acrolein-mediated damage, may be an effective means to halt degeneration after SCI. In this regard, utilizing acrolein scavengers to neutralize acrolein has shown some promising results including the reduction of post-SCI damage and additional neuroprotective effects. Aiming to improve the efficacy of several known scavengers, such as hydralazine and phenelzine, we have identified dimercaprol, a FDA approved drug for metal poisoning, as an alternative acrolein scavenger. Unlike hydralazine and phenelzine, which possess hydrazine groups, dimercaprol has thiol functional groups that could bind and trap acrolein. In this study, we have confirmed that dimercaprol can indeed react with acrolein through thiol groups in a non-biotic condition using Nuclear Magnetic Resonance (NMR) spectra. In fact, dimercaprol possesses two thiol groups with the potential for binding acrolein at the carbon-carbon double bond and the aldehyde group. The acrolein-scavenging ability was further examined in vitro on a PC-12 cell line. Dimercaprol at non-toxic concentrations could offer dose-dependent alleviation of acrolein-mediated PC-12 cell death based on WST-1, LDH and trypan blue cell viability assays. Interestingly, the necessary dosage of dimercaprol required to reduce cell death by 50 percent is substantially less than that of hydralazine, indicating it offers a higher efficacy for acrolein scavenging. This is also consistent with the fact that dimercaprol possesses two acrolein binding groups while hydralazine only has one. In addition, we have also shown that dimercaprol, at a safe dose of 5 mg/kg, can significantly lower acrolein levels in damaged spinal cord tissue in a rat contusive SCI model. Furthermore, dimercaprol application mitigated both motor deficits based on BBB score and sensory dysfunction based on Von Frey mechanical stimulation assessment. Taken together, these data suggest that dimercaprol may be an effective acrolein scavenger and neuroprotective agent in neuronal trauma.

Disclosures: R. Tian: None. R. Shi: None.
Title: Deleting PDE11A4 improves efficacy of the mood stabilizer lithium

Authors: *M. P. KELLY*¹, B. IBRAHIM², K. BISHARA², W. CAPELL², J. FISHER², N. PATEL², G. PATHAK²;
¹Pharmacology, Physiol. & Neurosci., ²Univ. of South Carolina Sch. of Med., Columbia, SC

Abstract: Lithium is a first line mood stabilizer for patients with bipolar disorder, but for unknown reasons approximately half of all patients do not respond to lithium. Lithium responsiveness has been genetically associated with single nucleotide polymorphisms (SNPs) in Phosphodiesterase 11A (PDE11A), a unique enzyme that breaks down cAMP and cGMP and is enriched in the hippocampus. These PDE11A SNPs are intronic, which suggests they may influence expression levels. Therefore, we determined if decreasing expression of PDE11A4, the isoform in brain, was sufficient to increase lithium responsivity in mice. Here, we show that BALB/cJ mice, which respond poorly to lithium, have significantly increased PDE11A4 protein expression relative to C57BL/6J mice, which respond well to lithium. This elevation in PDE11A4 protein occurs within both cytosolic and membrane compartments of dorsal hippocampus (DHIPP) but only the membrane compartment of ventral hippocampus (VHIPP). This compartment-specific difference in PDE11A4 expression appears to be related to a nonsynonymous coding SNP at amino acid 499, which falls within the PDE11 GAF-B protein-protein binding domain. *In vitro* studies conducted in COS1 cells show that, relative to the C57BL/6J 499A sequence, the BALB/cJ 499T sequence promotes aggregation of PDE11A4 (a putative readout of homodimerization). Further, relative to 499A, 499T shifts PDE11A4 from the cytosol to the membrane (a putative readout of binding partner interactions), perfectly replicating the differences in PDE11A4 expression noted within the VHIPP of BALB/cJ vs. C57BL/6J
mice. To determine if decreasing PDE11A4 expression is sufficient to increase lithium responsiveness, we tested the effect of chronic lithium treatment in PDE11A wild-type (WT) and knockout (KO) mice. Consistent with the fact that lower PDE11A4 expression correlated with better lithium responsiveness across mouse strains, we found that PDE11A KO mice given 0.4% lithium chow for 3 weeks exhibited greater lithium responsivity relative to WT littermates in tail suspension, an anti-depressant predictive assay, and in amphetamine hyperlocomotion, an anti-manic predictive assay. The ability of PDE11A4 to regulate lithium responsiveness may be related to IL6, a pro-inflammatory cytokine that has been implicated in mood disorders. C57BL/6J and PDE11A KO mice demonstrated elevated IL6 protein expression in hippocampus relative to BALB/cJ and PDE11A WT mice, respectively. Together, these findings show that PDE11A negatively regulates lithium responsiveness, suggesting that genetic associations found in patients with bipolar disorder may be functionally relevant.

Disclosures: M.P. Kelly: F. Consulting Fees (e.g., advisory boards); Deallus, Asubio. B. Ibrahim: None. K. Bishara: None. W. Capell: None. J. Fisher: None. N. Patel: None. G. Pathak: None.

Poster

502. Major Mental Disorders: Experimental Therapeutics

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 502.02/J30

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

Support: Stanley Medical Research Institute

Title: Development of kinome-wide and isoform selective inhibitors of GSK3α and GSK3β for the treatment of psychiatric disorders

Authors: *M. C. LEWIS1, F. F. WAGNER1, J. GALE1, A. J. CAMPBELL1, J. SACHER1, D. WALPITA2, D. FEI1, M. WEIWER1, L. ROSS1, A. J. HEYNEN4, L. STOPPEL3, M. WALK1, S. NGUYEN1, D. BARKER1, F. AN1, M. PALMER1, S. J. HAGGARTY5, M. BEAR4, K. STEGMAIER3, E. SCOLNICK1, Y.-L. ZHANG1, J. Q. PAN1, E. B. HOLSON1;

1Stanely Ctr., 2Ctr. for Sci. of Therapeut., Broad Inst., Cambridge, MA; 3Dana Farber Cancer Inst., Boston, MA; 4Picower Inst. for Learning and Memory, MIT, Cambridge, MA; 5Neurol., Massachusetts Gen. Hosp., Boston, MA

Abstract: The serine/threonine kinase Glycogen Synthase Kinase-3 beta (GSK3), part of the WNT signaling pathway, has been implicated in multiple human disorders including
neurological and psychiatric disorders. A growing number of direct genetic associations for WNT signaling have been established in bipolar disorder, schizophrenia and autism. In addition, a clear role for GSK3 in neuronal cell development and neuroplasticity has also been demonstrated by several groups. Importantly, in pre-clinical studies, the rapid anti-depressant effects of ketamine depend on GSK3β signaling, suggesting a therapeutic potential for GSK3β inhibitors in an acute setting in treatment resistant depression. It was also demonstrated in pre-clinical studies that potent GSK3β inhibitors may be efficacious in models of lithium insensitivity. These data suggest therapeutic potential of GSK3β inhibitors across a number of indications. While numerous GSK3α/β inhibitors are reported, none possess a desirable kinase-wide selectivity profile nor single isoform selectivity and suitable pharmacokinetic properties required of a CNS drug. Moreover, GSK3 inhibitors are known to increase β-catenin levels which has oncogenic potential. Therefore, we set out to identify small-molecule isoform selective GSK3α and GSK3β inhibitors to delineate the biological function of each isoforms and their potential use in a variety of disorders including psychiatric and neurological disorders.


Poster

502. Major Mental Disorders: Experimental Therapeutics

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 502.03/J31

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

Support: Stanley Medical Research Institute

Title: Development of b-arrestin biased D2R antagonists for the treatment of schizophrenia

Authors: *A. A. AMAYA1, M. WEIWER1, J. GALE1,2, M. LEWIS1, J. Q. PAN1, Y.-L. ZHANG1,2, Q. XU1,2, L. LI1,2, A. SKEPNER1,2, M. WALK1, D. FEI1,2, F. A. SCHROEDER3, J. M. HOOKER3, G. C. VAN DE BITTNER3, K. DENNEHY1,4, L. DORDEVIC1, S. NGUYEN1, F. F. WAGNER1, M. PALMER1, E. SCOLNICK1, E. B. HOLSON1; 1Stanley Ctr. for Psychiatric Res., Broad Inst. of MIT and Harvard Med. Sch., Cambridge, MA; 2Ctr. for the Develop. of Therapeut., Broad Inst. of MIT/Harvard, Cambridge, MA; 3Athinoula
Abstract: Schizophrenia is a devastating disease that affects 1-2% of the world’s population with limited progress in the development of new therapeutics. Current antipsychotic drugs all target the dopamine D2 receptor (D2R) as part of their pharmacology. D2R belongs to the superfamily of G-protein coupled receptors (GPCRs) and signal through the second messenger cAMP in a GPCR dependent manner and also through b-arrestin in a GPCR independent manner. All current antipsychotic drugs antagonize the D2R/b-arrestin pathway, while demonstrating mixed pharmacology at the D2R cAMP pathway (i.e. agonist (Aripiprazole) or antagonist (Clozapine)). Recent evidence suggests that the antagonist activity at b-arrestin is responsible for the antipsychotic effect while modulation of the cAMP pathway could be responsible for extrapyramidal side effects. Amphetamine induced hyperactivity (AIH) studies recently reported by the Caron lab (successfully reproduced in our lab) suggest that the psychotic phenotype is dependent on b-arrestin2 and support the development of b-arrestin biased D2R antagonists for the treatment of schizophrenia. We have now developed a toolkit of b-arrestin biased antagonists that will help us investigate the contribution of each signaling pathway downstream of D2 on both antipsychotic efficacy and motoric side effects.


Poster

502. Major Mental Disorders: Experimental Therapeutics

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 502.04/J32

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

Support: MH083729

COGNITO

Title: Limbic regulation of prefrontal glutamate and dopamine release is mediated by stimulation of cortical alpha7 nicotinic receptors
Authors: *V. VALENTINI*, D. M. BORTZ, V. PERRA, G. P. PIETRO, D. PHENIS, G. DI CHIARA, J. P. BRUNO;

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Abstract: Dysregulations within a neural system containing the nucleus accumbens (NAC), basal forebrain (BF), medial dorsal thalamus, and prefrontal cortex (PFC) contribute to the cognitive deficits seen in several neuropsychiatric disorders (e.g. schizophrenia, ADD, drug addiction). Previously we demonstrated that stimulation of the NAC shell with NMDA evoked ACh release in PFC and that 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 NMDA activation elevates both PFC choline and glutamate levels. Here we determined if a) NAC-evoked glutamate levels were also seen using more traditional microdialysis methods; b) NAC activation also increased DA release in PFC; and c) the relative contributions of nicotinic receptor subtypes to glutamate and DA release.

Rats were implanted with an infusion cannula into the NACshell and either a biosensor or microdialysis probe in the ipsilateral mPFC. NMDA was infused and extracellular levels of glutamate and DA were measured. In a separate group, the role of nicotinic (α7, α4β2) receptors in this stimulated release was determined following local perfusions (1.0 or 10.0 μM) of mecamylamine (MEC), α-bungarotoxin (α-BGT), DHβE, or galantamine (3.0 mg/kg). The TTX (0.1 μM) dependency of basal and evoked glutamate and DA release was also determined. Infusions of NMDA (0.05, 0.15, 0.30 μg/0.5 μL) produced dose dependent increases in glutamate as measured with the sensor (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 (α7) as MEC or α-BGT blocked the increases. Blockade of α4β2 receptors resulted in partial reductions. Finally, TTX blocked stimulated release of glutamate and DA but only affected basal DA. The results confirm, using two methods, the limbic stimulation of prefrontal glutamate release. The 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. Evoked release of glutamate and DA are secondary to the release of ACh and a subsequent activation of local nicotinic (α7) receptors on glutamate and DA terminals.

Title: Positive allosteric modulators of the alpha7 nicotinic acetylcholine receptor potentiate glutamate levels in prefrontal cortex \textit{in vivo}

Authors: *D. M. BORTZ*, B. A. UPTON, J. D. MIKKELSEN, J. P. BRUNO

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Abstract: Positive allosteric modulators (PAMs) of the alpha7 nicotinic acetylcholine receptor (α7nAChR) may have potential as cognition-enhancing drugs. PAMs better preserve the nature of endogenous cholinergic transmission than direct agonists because agonists stimulate receptors independent of presynaptic activity. PAMs' effects depend upon cholinergic activity, with the extent of potentiation interacting with levels of ACh/choline in cellular models. Since this effect has only been demonstrated \textit{in vitro}, we determined if it would hold true \textit{in vivo} by using an assay designed to a) dose-dependently evoke glutamate and choline release in PFC from awake rats and b) improve rodent performance on an attention task. Importantly, the glutamate release depends upon cholinergic activity at the α7nAChR. We tested the hypothesis that two α7nAChR PAMs (type I and II) would potentiate glutamate release in the PFC, \textit{in vivo}, following afferent stimulation but not under basal conditions, and that the magnitude of this potentiation would vary as a function of PAM type and choline levels in PFC. Adult male Wistar rats received infusion cannulae into their nucleus accumbens shell (NaccSh) and a choline- or glutamate-sensitive biosensor in their ipsilateral medial prefrontal cortex (mPFC). On each of 3 consecutive test days, rats were infused with NMDA (aCSF, 0.05, or 0.30 µg/0.5 µL) 40 minutes after a systemic injection of either AVL3288 (type I, 5% DMSO vehicle, 1, or 3 mg/kg) or PNU120596 (type II, 5% DMSO vehicle, 3, or 9 mg/kg), and extracellular levels of glutamate were measured. Infusion of aCSF into NaccSh did not evoke choline release in mPFC, and neither PAM potentiated glutamate release under these conditions. The low dose of AVL3288 (1 mg/kg) potentiated glutamate release after both the low (0.05 µg; 24.2% increase) and high (0.30 µg; 84.7%
increase) doses of NMDA, relative to NMDA + vehicle. The high dose of AVL3288 (3 mg/kg) had no effect on the low dose of NMDA, and inhibited glutamate release after the high dose of NMDA (64.2% decrease). The low dose of PNU120596 (3 mg/kg) failed to significantly potentiate either dose of NMDA. The high dose of PNU120596 (9 mg/kg) potentiated glutamate release after the low dose of NMDA (211%), relative to vehicle, but had no effect on the high dose of NMDA. These *in vivo* data extend previous *in vitro* results by revealing that the extent of PAM potentiation depends upon level of afferent activity at local α7nAChRs, and that there are important differences in the potentiation of type I vs. II PAMs. Thus, PAMs have potential as replacements for agonists, but comparisons in behavioral studies are needed to determine whether type I or II PAMs effectively enhance cognition.

**Disclosures:** D.M. Bortz: None. B.A. Upton: None. J.D. Mikkelsen: None. J.P. Bruno: None.

**Poster**

**502. Major Mental Disorders: Experimental Therapeutics**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program/#Poster#:** 502.06/334

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

**Support:** RO1MH91130

**Title:** Dose dependent effects of delta-9-tetrahydrocannabinol on correlates of schizophrenia in the sub-chronic PCP rat model

**Authors:** *A. SEILLIER, S. A. PEREZ, A. A. MARTINEZ, D. J. LODGE, A. GIUFFRIDA; Pharmacol., UTHSCSA, San Antonio, TX

**Abstract:** The “cannabinoid hypothesis” of schizophrenia postulates that an over-active endocannabinoid system may contribute to the etiology of this pathology. However, we recently challenged this hypothesis by showing that social withdrawal, a behavioral correlate of the negative symptoms of the disease, resulted from deficient, rather than over-active, endocannabinoid transmission. This discrepancy might arise from the divergent effects of cannabis, which negatively affects the course and expression of psychosis, versus those of endocannabinoids. In this study, we tested whether the beneficial effects of endocannabinoid-mediated CB1 activation on social withdrawal in phencyclidine (PCP)-treated Wistar rats (5 mg/kg, b.i.d. for 7 days, followed by a washout period) could also be observed after administration of the psychoactive ingredient of the Cannabis plant, delta-9-tetrahydrocannabinol...
(THC). To determine whether these observations could be generalized to positive symptoms, we also assessed the effects of THC (0, 0.1, 0.3, 1.0 mg/kg, i.p.) on: 1) motor activity induced by d-amphetamine (0.5 mg/kg, i.p.), and 2) dopamine neuron population activity in the ventral tegmental area (VTA). The brains from d-amphetamine-treated animals (immediately after the motor activity test) were further processed for endocannabinoid measurements. Systemic administration of THC reversed PCP-induced social withdrawal at the lowest dose tested, whereas it significantly reduced social interaction in saline-treated rats (at all the doses tested with the exception of 0.3 mg/kg). Although PCP-treated rats did not differ from saline-treated controls in the motor response elicited by d-amphetamine (irrespective of THC treatment), they showed a 2.3 fold increase of anandamide (AEA) levels in the nucleus accumbens. This AEA elevation was completely reversed by THC (at all the doses tested), which had no effect per se in saline-control rats. Interestingly, we found a robust decrease in the number of VTA dopamine neurons firing spontaneously in PCP-treated rats. THC at the lowest, but not highest, dose was able to normalize this effect in PCP-treated rats, but produced a deficit on its own at the lowest dose in saline-treated rats. Taken together, these data suggest that in contrast to high doses, low doses of THC have beneficial effects on correlates of both negative and positive symptoms of schizophrenia. This observation might shed some light on the controversial hypothesis of marijuana use as self-medication in schizophrenia patients. Supported by RO1MH91130.


Poster

502. Major Mental Disorders: Experimental Therapeutics

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 502.07/J35

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

Title: The effects of PDE4 and PDE10 inhibition on auditory processing in mice

Authors: *L. SCOTT1, C. BUZBY2, Z. HUGHES1;
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Abstract: Sensory processing abnormalities have been observed in numerous psychiatric diseases, including schizophrenia. Auditory-evoked potentials (AEPs) have been used to assess these sensory processing deficits in schizophrenia patients as well as in rodent models of schizophrenia. In the present study AEPs were recorded from the frontal cortex of freely moving adult male C57BL/6J mice, using a paired pulse paradigm. Utilizing a within subject crossover
design, the effects of the novel PDE4 inhibitor, ABI-4 on AEPs were assessed and compared to those of the clinically prescribed antipsychotics, risperidone and haloperidol. The failed clinical candidate PDE10 inhibitor, PF-02545920 was included as a putative negative control compound. The PDE4 inhibitor ABI-4 (0.01-1.0 mg/kg, s.c.) caused a dose-dependent increase in the AEP in response to the first tone of each pair presented (S1) while also decreasing the response to the second tone presented (S2), resulting in enhanced auditory gating (vehicle S2/S1= 0.41 ± 0.04 ; ABI-4 (0.1 mg/kg, s.c.) S2/S1 = 0.26 ± 0.02; P<0.001). Fast Fourier transform (FFT) also revealed a dose-dependent decrease in EEG power across all frequency bands. Similarly to PDE4 inhibitors, risperidone (1.0-3.0 mg/kg, s.c.) and haloperidol (1.0-3.0 mg/kg, s.c.) were also shown to increase auditory gating in a dose-dependent manner, however this increase in gating was mediated through an increase the S1 AEP while the S2 response was unchanged (vehicle S2/S1= 0.43 ± 0.02 ; risperidone (3.0 mg/kg, s.c.) S2/S1 = 0.34 ± 0.02 ; haloperidol (3.0 mg/kg, s.c.) S2/S1 = 0.29 ± 0.02). Risperidone and haloperidol also cause a decrease in delta (1-4Hz) and theta (4-12Hz) power, but did not affect gamma (30-100Hz) power. The PDE10 inhibitor PF-02545920 had no effect on either the S1 or S2 AEP or EEG power across any frequency band. Finally, the ability of PDE10 and PDE4 inhibition to enhance risperidone-induced increases in sensory processing was also assessed. This study increases confidence that this assay may prove useful in supporting other pre-clinical programs targeting sensory processing deficits, as well as supporting the use of AEP as an electrophysiological end-point in clinical proof of mechanism and potentially proof of concept studies.

Disclosures:  L. Scott: A. Employment/Salary (full or part-time); Pfizer INC. C. Buzby: A. Employment/Salary (full or part-time); Pfizer INC. Z. Hughes: A. Employment/Salary (full or part-time); Pfizer INC.

Poster

502. Major Mental Disorders: Experimental Therapeutics

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 502.08/J36

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

Support: NIH R01MH094358

Title: Phosphorylation of Heterochromatin Protein 1 (HP1gamma) and H3S10 by antipsychotics

Authors: *B. M. FEINER1, K. A. CHASE1,2, J. MELBOURNE1, C. ROSEN1, R. P. SHARMA1,3;
Abstract: Background: Global levels of different epigenetic modifications have been found to be altered in patients with schizophrenia. We have previously reported altered levels of histone acetylation and methylation in schizophrenia subjects. However, most psychotropic drugs such as antipsychotics act through membrane receptors that are commonly linked to kinase cascades, which reach into the nucleus and phosphorylate nuclear proteins such as histones, transcription factors and heterochromatin proteins. Heterochromatin protein 1 (HP1) is proposed to encourage the spreading of restrictive chromatin domains through the binding of methylated H3K9, and acts as a bridge to adjacent nucleosomes, either binding to itself or through interplay with other stabilizing heterochromatic factors. However, phosphorylation of the γ isoform of HP1 causes it to disengage and encourages disassembly of heterochromatin, resulting in a transcriptionally facilitative environment. Methods: Human SW872 cells, selected for their expression of D2 receptor protein and mRNA, were cultured in the presence of Clozapine, Haloperidol or Forskolin for 40 and 120 minutes, at which point their proteins were extracted in the presence of phosphatase inhibitors. Levels of phosphorylated HP1γ and H3S10 were then determined via Western blot and normalized to β-actin. Results: We found that treatment with forskolin increases the levels of HP1γ, as would be predicted by its effects on cAMP levels and PKA pathway. Clozapine and Haloperidol increase levels of both HP1γphos and H3S10phos. We can demonstrate a dose and duration effect. Discussion: Phosphorylation events, while transient and reversible, upon repetition can eventually lead to long-term changes in the cell. For example, treatment with Clozapine has been shown to increase levels of phosphorylated MEK in the prefrontal cortex of rats. The MEK/ERK pathway is well-characterized, and includes a myriad of acute and long-term neuronal effects, such as potassium channel activation in the acute phase and transcription factor activation in the longer term. Acute activation of kinase cascades such as MEK/ERK could lead to lasting alterations in neuronal function that could explain the efficacy seen in antipsychotic drugs only after weeks of treatment.

Title: Glycinamide blocks MK-801-induced hyperlocomotion in an inverted U-shaped fashion in an animal model of positive-like symptoms of schizophrenia

Authors: *E. BASURTO, K. L. HOFFMAN, O. GONZALEZ-FLORES;
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Abstract: Experimental evidence suggests that hypofunction of the NMDA receptor participates in the pathophysiology of schizophrenia. Pharmacological attempts to enhance NMDA receptor activity involve the obligate co-agonists glycine, D-serine and D-cycloserine, which bind at the glycine modulatory site (GMS) of this receptor. In many, but not all studies, these compounds show antipsychotic effects. Inconsistent effects of these compounds might be explained by their low ability to cross the blood-brain barrier (BBB). Glycinamide is a synthetic precursor of glycine that readily crosses the BBB. In previous studies in the rat and rabbit, we demonstrated that glycinamide prevented MK-801-induced hyperlocomotion and deficits in object recognition memory. In order to characterize dose-dependent effects of glycinamide, in the present study we examined the effects of three doses of glycinamide (37, 56, 112 mg/kg, ip.) on MK-801-induced hyperlocomotion in rats, an animal model that resembles positive symptoms of schizophrenia.

Disclosures: E. Basurto: 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.; # 129381. K.L. Hoffman: None. O. Gonzalez-Flores: None.

Poster

502. Major Mental Disorders: Experimental Therapeutics

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 502.10/J38

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

Title: NSX-0527: A novel M1/M4 selective muscarinic agonist with antipsychotic and cognition-enhancing properties

Authors: J. C. OCKULY, J. D. BECK, S. A. HANSON, *M. L. HENDRICKSON;
Neurosci., NeuroSolis, Inc., Madison, WI
Abstract: Schizophrenia affects nearly 2.5 million Americans, comprising 1.1% of the adult population. Many treatments are approved, but pharmacotherapy of the disease remains problematic. Antipsychotics do provide some benefit in mitigating the positive symptoms of the disease (delusions and hallucinations), but have little effect on the negative symptoms (emotional blunting) and cognitive symptoms (impaired learning and memory). However, these compounds produce drowsiness, weight gain, and metabolic disruption, which can lead to significant morbidity and contribute to decreased medication adherence. Research into muscarinic agonists has suggested that pharmacologically balancing dopaminergic and cholinergic activity via stimulation of M4 muscarinic receptors may be as effective in treating schizophrenia as currently approved therapies. Several muscarinic agonists have been investigated in early clinical trials with the target indication of Alzheimer’s disease. One of these, the M1/M4-preferring agonist xanomeline showed efficacy in AD patients. Interestingly, in addition to cognitive benefits, the compound reduced vocal outbursts, suspiciousness, delusions, agitation, and hallucinations. NSX-0527 is an M1/M4-selective orthosteric muscarinic agonist showing good bioavailability (~75%) and brain penetration (~60%), excellent metabolic stability, and a half-life of approximately one hour in rats. It was investigated in three behavioral antipsychotic assays: reversal of apomorphine-induced climbing, reversal of MK-801- and amphetamine-induced hyperlocomotion, and inhibition of conditioned avoidance response. NSX-0527 compared favorably to xanomeline as well as olanzapine without producing the classic antipsychotic side effect of hyperprolactinemia. NeuroSolis has also developed an NSX-0527 formulation that minimizes the peripheral effects of high doses. Finally, mice dosed with NSX-0527 demonstrated improved memory in the novel object recognition test, suggesting that NSX-0527 has the potential to be a first-in-class treatment that both reduces positive symptoms and enhances cognition.


Poster
502. Major Mental Disorders: Experimental Therapeutics

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM
Title: Pharmacological blockade of Lingo-1 in combination with olanzapine administration reverses phencyclidine induced effects on dendritic morphology, cognitive performance and locomotor activity

Authors: J. L. ANDREWS1,2,3, R. P. SULLIVAN4, K. A. NEWELL1,2,3, X.-F. HUANG1,2,3, F. FERNANDEZ-ENRIGHT1,3,5;
1Illawarra Hlth. and Med. Res. Inst., University of Wollongong, Australia; 2Fac. of Science, Med. and Hlth., University of Wollongong, Australia; 3Schizophrenia Res. Inst., Sydney, Australia; 4ARC Ctr. of Excellence for Electromaterials Science, Intelligent Polymer Res. Inst., University of Wollongong, Australia; 5Sch. of Psychology, Fac. of Social Sci., University of Wollongong, Australia

Abstract: Background and Aims: Myelination dysfunction is one of the strongest hypotheses implicated in schizophrenia pathophysiology. Interestingly, myelination peaks during late adolescence, coinciding with the onset of schizophrenia. Lingo-1, a transmembrane signal-transducing molecule expressed on oligodendrocytes and neurons, is a potent negative regulator of oligodendrocyte differentiation, axonal growth and myelination. Since myelination and neuronal outgrowth disturbances lead to cognitive dysfunction, and considering the involvement of Lingo-1 in these events, we have investigated the effects of pharmacological inhibition of Lingo-1 as a novel treatment for schizophrenia in these processes. Methods: Adolescent male Sprague Dawley rats (4 weeks) were injected subcutaneously with either saline vehicle or PCP (10 mg/kg, Sigma) for a total of 8 days. On the third day, rats (n=12/group) were concurrently treated for 5 days with either olanzapine (Olz) (oral administration by cookie dough 1 mg/kg/day, 3 times/day), vehicle cookie dough and/or an anti-Lingo-1 functional antibody ab23631 (Abcam, UK), (intracerebroventricular injection: i.c.v), or saline (i.c.v), with surgery for intracranial cannula implantation performed one week prior. Behavioral testing (locomotor activity and novel object recognition) was performed prior to sacrifice. Dendritic morphology (assessed by Golgi staining) and protein expression levels of Lingo-1, and myelination marker myelin basic protein (MBP) were examined within the prefrontal cortex of the treated rats. Results: Locomotor activity was significantly increased in PCP treated rats (p=0.044) and both preference index and dendritic spine density were significantly reduced in PCP treated rats (0.006<p<0.014). Behavior and spine density were restored to near control levels in anti-Lingo-1/Olz treated rats (0.001<p<0.004). While Lingo-1 protein expression was increased by PCP.
administration (p=0.019), the combined anti-Lingo-1/Olz treatment had no effect of normalizing Lingo-1 protein levels (p=0.325). Myelination as measured by MBP levels, were not significantly altered by any of the treatments (p>0.05). Conclusions: This is the first study to show that increased locomotor activity, decreased preference index and decreased dendritic spine density, all induced by PCP can be reversed by a combined anti-Lingo-1/Olz therapy. We suggest that Lingo-1 may be a suitable target for the development of new future therapeutic treatments targeting the cognitive deficits of schizophrenia.


502. Major Mental Disorders: Experimental Therapeutics

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 502.12/340

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

Support: Brain and Behavior Research Foundation

Title: Pharmacotherapeutic potential of disrupting neuromodulation of hyper-dopaminergic neural activity in the co-morbid expression of schizophrenia and drug addiction

Authors: *T. COOMER, J. GALLEGOS, N. RAUSCHER, P. ELLO, K. SANDERS, R. DAS, E. OLESON; Univ. of Colorado Denver, Denver, CO

Abstract: Schizophrenia is a debilitating psychopathology that is exacerbated by patients showing a predilection for addictive behavior. The high co-morbidity between schizophrenia and drug addiction theoretically arises from a hyper-dopaminergic state in schizophrenia, pre-sensitizing the neural mechanisms that invigorate drug seeking. Due to recent technical advances, we can now directly test this theory by assessing the causal role of heightened dopamine (DA) release in behaviors relevant to the co-morbid expression of schizophrenia and addiction. Our research attempts to establish the causality of DA in eliciting a pro-psychotic response in a conditioned avoidance task, which is a classical screen with high predictive validity for determining the efficacy of anti-psychotic drugs. We then attempt to counteract that response pharmacologically. Historically, both typical and atypical antipsychotics target the dopamine D2 receptor, but a number of issues exist with these pharmacotherapies that result in poor compliance. We propose an alternative method of treatment that targets upstream modulators of
dopaminergic neurons in the mesocorticolimbic pathway that will potentially ameliorate both the schizophrenic symptoms, and drug-seeking behaviors. To achieve this, our group artificially induces a hyper-dopaminergic state in transgenic rats by utilizing Gq-coupled DREADD virus technology. We then systemically administer antagonists of the cannabinoid CB1 receptor and orexin OX1 receptor, which we have previously demonstrated to modulate DA neural activity. Preliminary results show a DREADD-induced hyper-dopaminergic state evokes a pro-psychotic response in a classic pharmacological screen, as well as increases locomotor activity and motivation for cocaine; whereas, an anti-psychotic response and reduced motivation for cocaine is observed when either the CB1 or OX1 antagonist drug is administered. Additionally, we have found that both of these effects can be amplified when the two drugs are administered concurrently. These results show promise for targeting upstream modulators of DA function in the treatment of co-morbid diagnoses of schizophrenia and drug addiction.


Poster

502. Major Mental Disorders: Experimental Therapeutics

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 502.13/J41

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

Support: OMHF

CIHR

Title: Positive allostery: A new approach to the treatment of schizophrenia

Authors: *R. P. DAYA¹, J. K. BHANDARI¹, S. K. KOONER¹, R. L. JOHNSON², R. K. MISHRA¹;
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Abstract: Antipsychotic medications are the first line of defense against schizophrenia and other severe neuropsychiatric illnesses. Unfortunately, antipsychotic treatments control only a subset of the symptoms and induce adverse side effects that can be more severe than the illness itself. It is clear that a new approach to treatment is needed to better treat and manage the symptoms of this complex neuropsychiatric illness. We have investigated the use of a positive dopamine D2 receptor allosteric modulator (PAOPA) in regulating receptor expression as a new approach to
ameliorating the positive, negative, and cognitive symptoms of schizophrenia. In contrast to current forms of antipsychotic medication, which antagonize dopamine binding, PAOPA enhances binding to the dopamine D2 receptor. Our previous studies suggest that positive allosteric modulation with PAOPA leads to subsequent receptor internalization of the dopamine D2 receptor, ultimately decreasing dopaminergic neurotransmission and regulating aberrant receptor expression. In the present study, PAOPA was tested for its therapeutic efficacy across a battery of tests (hyperlocomotion, social withdrawal, sensorimotor gating, novel object recognition, brain metabolic activity, 5-choice serial reaction time task) in the phencyclidine and amphetamine induced rat models of schizophrenia. PAOPA showed therapeutic efficacy in behavioural paradigms representing the positive, negative, and cognitive symptoms of schizophrenia. Interestingly, some behavioural indices that were ameliorated in the amphetamine model were not ameliorated in the PCP model, suggesting that the deficits induced by amphetamine and PCP--while behaviourally and phenotypically similar--are mechanistically different and that PAOPA’s effects are limited to certain mechanisms and systems. These studies provide insight into the use of positive allostery for the safe and effective treatment of schizophrenia.


Poster

502. Major Mental Disorders: Experimental Therapeutics

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 502.14/J42

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

Support: DFG (BR 3723/3-1)

Title: The ergoline 2-bromoterguride produces antipsychotic-like effects and mild hyperprolactinemia in rats

Authors: *J. BROSDA¹, E. A. TARLAND¹, R. T. FRANKE¹, H. H. PERTZ²; H. FINK¹;
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Abstract: Objectives: The therapy of schizophrenic patients with currently available antipsychotic drugs is of limited efficacy. 2-Bromoterguride, a dopamine D2 receptor partial agonist, has previously been shown to have antipsychotic-like activity in rats (reversal of
amphetamine-induced locomotion) without inducing adverse side effects (no changes in body weight/body fat composition and cataleptic behavior, respectively) [1]. The conditioned avoidance response (CAR) and the prepulse inhibition (PPI) of the acoustic startle response were conducted to verify the antipsychotic potential of 2-bromoterguride. In addition, the effect of 2-bromoterguride on prolactin release was investigated to screen for further potentially adverse side effects. Methods: In male Sprague-Dawley rats aged 10-15 weeks, the effects of 2-bromoterguride (0.1 and 0.3 mg/kg; i.p.) on (1) the avoidance behavior in the CAR test (two-way avoidance paradigm) 30, 90, 270min and 24h post-injection and on (2) apomorphine- or phencyclidine (PCP)-induced PPI-deficits were investigated. (3) At various time points after injection of 2-bromoterguride (1, 2, 4, 8h; 0.1 and 0.3 mg/kg; i.p.), the plasma prolactin concentration was determined by enzyme-linked immunosorbent assay (ELISA). Established antipsychotics (haloperidol, clozapine, aripiprazole) were used as positive controls. Results: Preliminary results show that 0.3mg/kg 2-bromoterguride significantly decreased CAR at 30 and 90min post-injection in a comparable manner to haloperidol and aripiprazole. Furthermore, 0.3mg/kg 2-bromoterguride antagonized apomorphine- but not PCP-induced sensorimotor gating deficits in the PPI-paradigm. 2-bromoterguride (0.3mg/kg) triggered a reduced prolactin release compared to the D2 receptor antagonist haloperidol (0.5 mg/kg). Conclusions: In the present study, the CAR test revealed that 2-bromoterguride, haloperidol and aripiprazole decreased avoidance levels, which is indicative of antipsychotic activity. Additionally, 2-bromoterguride ameliorated PPI-deficits induced by the D2 agonist apomorphine but not those induced by the non-competitive NMDA antagonist PCP, which is in agreement with the effect of various atypical antipsychotics on drug-induced PPI-deficits. Besides, the mild hyperprolactinemia indicates the drugs’ effect on the pituitary, which is not as distinct as the effect of the full D2 antagonist haloperidol. Due to the in vitro properties [1] and the in vivo antipsychotic-like effects of 2-bromoterguride, the terguride derivative may be a promising candidate for the treatment of schizophrenia with a low risk to induce adverse side effects.


Poster

502. Major Mental Disorders: Experimental Therapeutics

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 502.15/J43

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

Title: Effects of chronic haloperidol treatment on the nigrostriatal dopamine system
Authors: D. GROOS1, *F. ZHENG2,3, C. P. MÜLLER3, C. ALZHEIMER1; 
1Dept. of Physiol. and Pathophysiology, 3Dept. of Psychiatry and Psychotherapy, 2Univ. of Erlangen-Nürnberg, Erlangen, Germany

Abstract: Haloperidol is a widely used, typical antipsychotic drug, whose therapeutic benefits and side effects in schizophrenic patients have been both linked to D2 dopamine receptor antagonism in different brain regions. In previous animal studies on chronic effects of haloperidol, the drug was typically given daily via intraperitoneal injection. Here, we re-evaluated the effect of chronic haloperidol on the nigrostriatal dopamine system in mice by taking advantage of subcutaneously implanted osmotic mini-pumps as a reliable method to achieve sufficiently high drug levels comparable to those of chronically treated patients. Adult C57Bl6 mice received haloperidol (0.5 mg/kg/d) or vehicle for 6 and 14 days, corresponding to the time points when the drug was effective or already fading, respectively. To determine the impact of chronically administered haloperidol on the electrical activity of dopaminergic neurons and on one of their target regions, we prepared brain slices from treated animals and performed whole-cell recordings from substantia nigra pars compacta (SNc) dopaminergic neurons as well as field potential recordings in dorsal striatum (DS). Chronic haloperidol treatment for 14 days reduced the intrinsic excitability of SNc dopaminergic neurons, as indicated, firstly, by an increased number of silent dopaminergic neurons, with resting membrane potentials shifted to more negative values, and, secondly, by a reduced firing frequency in neurons displaying spontaneous activity. The action potential waveforms of spontaneously active dopaminergic neurons from haloperidol-treated mice showed a significantly stronger afterhyperpolarization when compared to that of neurons from vehicle-treated mice. Interestingly, the synaptic field potentials in the DS, the major projecting area of SNc dopaminergic neurons, displayed time-dependent changes in basal transmission, with a reduction after 6 days and an enhancement after 14 days of haloperidol treatment. Independent of the duration of its application, haloperidol markedly enhanced the use-dependent inhibition of synaptic field potentials during trains of 40 stimuli at 25 Hz. Our data show that chronic haloperidol exerts a dampening effect on the nigrostriatal dopamine system in that it reduces the number of spontaneously active neurons and slows their firing rate. Furthermore, chronic haloperidol appears to have complex actions on basal and plastic synaptic properties in the DS, some of which appear to critically depend on the length of drug application.


Poster
502. Major Mental Disorders: Experimental Therapeutics

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM
Title: Green tea polyphenol, EGCG, attenuates phencyclidine-induced HSP70 expression and hyperlocomotion in the rat

Authors: *A. S. DARVESH*1, W. J. GELDENHUYS1, P. SADANA1, C. PAXOS1, A. PRUS2, H. BERGSTROM3, C. K. MESHUL4, S. P. BERGER5;
1Northeast Ohio Med. Univ. (NEOMED), Rootstown, OH; 2Northern Michigan Univ., Marquette, MI; 3NIH, Rockville, MD; 4Oregon Hlth. & Sci. Univ., Portland, OR; 5Skybridge Pharmaceuticals Inc., Portland, OR

Abstract: Noncompetitive N-methyl-D-aspartate (NMDA) antagonists such as phencyclidine (PCP) and ketamine produce psychotic behavior in humans and are used extensively as pharmacological models of schizophrenia in rodent pre-clinical studies. NMDA antagonists induce behavioral effects such as ataxia and hyperlocomotion. NMDA antagonists also produce neuronal injury and increase expression of heat shock protein 70 (HSP70), a marker for cellular injury and neurotoxicity, in the rat cingulate cortex. It has been hypothesized that neuronal circuits and biochemical mechanisms that clinically mediate PCP-induced psychotic behavior may be similar to those involved in the neurotoxic and behavioral effects of NMDA antagonists. Oxidative stress, produced by both reactive oxygen and nitrogen species, as well as inflammation, have been strongly implicated in the pathophysiology of schizophrenia. Thus both antioxidants and anti-inflammatory agents are being investigated for their therapeutic potential in schizophrenia. The present study investigated the effects of epigallocatechin-3-gallate (EGCG), a polyphenol present in green tea, which has potent antioxidant and anti-inflammatory properties, as well as the ability to inhibit the enzyme inducible nitric oxide synthase (iNOS), on PCP-induced HSP70 expression and hyperlocomotion. We also investigated the effect of aminoguanidine (AG), a selective iNOS inhibitor, on the aforementioned PCP effects. Adult, male, Sprague-Dawley rats showed a dose-dependent and significant (p<0.05) increase in HSP70 expression in the cingulate cortex, 24 hrs after PCP (10, 20, 30 mg/kg, i.p.) administration as measured by western blot analysis. Pre-treatment with EGCG (100 mg/kg, i.p.) or AG (100 mg/kg, i.p.) 30 min earlier, significantly (p<0.05) attenuated the PCP (30 mg/kg, i.p.)-induced HSP70 expression. PCP (5 mg/kg, i.p.)-induced hyperlocomotion in rats, measured for 60 min, was significantly (p<0.05) blocked by EGCG (100 mg/kg, i.p.) or AG (100 mg/kg, i.p.) pre-treatment, 30 min prior to PCP administration. These results support the hypothesis that iNOS mechanisms contribute to the acute neurotoxic and behavioral effects of PCP. These results also suggest that the iNOS inhibitory effect of tea polyphenols may contribute to their potential antipsychotic properties. It has also been hypothesized that naturally occurring dissociative episodes in post-traumatic stress disorder (PTSD) occur by mechanisms similar to those produced by dissociative drugs such as PCP. Thus the aforementioned results may be relevant to PTSD therapy as well.
Poster

502. Major Mental Disorders: Experimental Therapeutics

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 502.17/J45

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

Support: RDA Grant PJ011582032015

NRF Grant 2010-0021521

Title: Effects of tianeptine on adults offspring rats exposed prenatally stressed rats: evaluation prenatally preventive antipsychotic and antidepressant drug treatment

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Abstract: Exposing a pregnant female to stress during the critical period of fetal brain development is a risk factor for the development of psychiatric disorders in offspring. In this study, a repeated variable stress paradigm was applied to pregnant rats during the last week of gestation. The effects of the antidepressant tianeptine on prenatally stressed (PNS) rats were investigated in terms of behavioral and protein expression analyses. Many of the forced-swimming, open-field, and social interaction test parameters decreased in the PNS rats compared with those in non-stressed offspring, but the behavioral changes recovered after tianeptine treatment. Western blot and immunohistochemical analyses of the prefrontal cortex revealed that downregulation of several neurodevelopmental genes in the PNS rats recovered after tianeptine treatment. These findings demonstrate that downregulation of several genes in the PNS rats may have affected the subsequent behavioral changes, and that these phenomena recovered following tianeptine treatment. Our results suggest that tianeptine may reduce the incidence of prenatal stress related-psychiatric disorders, such as depression and schizophrenia.


Poster
Title: Embryonic stem cell transplants as a therapeutic strategy in a rodent model of schizophrenia

Authors: *J. J. DONEGAN¹; J. TYSON²; S. ANDERSON²; D. LODGE¹;
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Abstract: Schizophrenia is a devastating psychiatric disorder characterized by positive symptoms, such as delusions and hallucinations, negative symptoms, like reduced social interaction and anhedonia, and cognitive deficits. The dopamine hypothesis of schizophrenia suggests that enhanced activity in the mesolimbic dopamine system underlies symptoms of the disorder. However, no primary pathology exists in the dopamine system of schizophrenic patients and currently prescribed antipsychotic medications, which target dopamine receptors, only alleviate positive symptoms of the disorder. Previously, our lab and others have demonstrated that hyperactivity in brain regions upstream of the dopamine system, such as the ventral hippocampus (vHipp), are responsible for the increase in dopamine cell activity and schizophrenia-like symptoms in the methylazoxymethanol acetate (MAM) model of schizophrenia. Schizophrenic patients show increased vHipp activity and reduced parvalbumin (PV) and somatostatin (SST) interneurons in this brain region. Therefore, we hypothesized that restoring interneuron function in the vHipp would reverse schizophrenia-like deficits in the MAM model. To test this hypothesis, we used a mouse embryonic stem cell line containing dual reporters (Lhx6::GFP and Nkx2.1::mCherry) to grow interneuron populations enriched for either PV or SST subtypes. Cells were sorted using flow cytometry, then injected into the vHipp of MAM rats. After 30 days, a sufficient time for the cells to migrate and integrate into the existing circuitry, we performed a battery of behavioral assays to measure positive, negative and cognitive symptoms, including latent inhibition, social interaction, and attentional set-shifting. In addition, we used *in vivo* extracellular recordings to assess pyramidal cell firing in the vHipp and dopamine population activity in the ventral tegmental area (VTA). We found that SST-positive interneurons attenuate deficits in reversal learning and reduce dopamine population activity. Excitingly, the PV-positive transplants also improve reversal learning and normalize dopamine cell activity in MAM rodents, but also reversed deficits in extradimensional set-shifting and...
social interaction. These results suggest that PV interneuron transplants may be an effective treatment strategy for the positive, negative and cognitive symptoms of the disorder.


Poster

502. Major Mental Disorders: Experimental Therapeutics

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 502.19J47

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

Support: NIH K08

Title: The glutathione cycle: an access point to target neural glutamate and oxidative stress

Authors: *T. W. SEDLAK, M. KOGA, C. HIGGS, P. TALALAY, A. SAWA; Johns Hopkins Sch. of Med., Baltimore, MD

Abstract: Glutathione is the principal cellular antioxidant and alterations, predominantly decreases, are found in a variety of neuropsychiatric conditions including schizophrenia and Alzheimer’s disease. Glutathione, is one third glutamate and present at 0.5-3 millimolar intracellular concentrations in brain, participating in antioxidant defense and drug detoxification. We find that glutathione is a physiologically relevant reservoir of glutamate, and increasing or decreasing liberation of glutamate from the glutathione cycle can, respectively, increase or decrease miniature excitatory post synaptic potential (mEPSC) frequency in rat primary cortical neurons. Sulforaphane, a plant derived isothiocyanate, augmented protein expression of GCL, the rate limiting enzyme of the glutathione cycle, and increased glutathione levels in cultured neurons. In a translational study in human subjects, sulforaphane demonstrated increases in monocyte intracellular glutathione (p=0.08, n=7). Our findings bridge independent findings of glutathione and glutamatergic dysfunction in neuropsychiatric disorders, and suggest tools with which glutathione and glutamate levels might be targeted.


Poster

503. Cognition and Anxiety: Human Studies
Abstract: Fear learning entails establishing an association between a neutral stimulus (conditioned stimulus, CS) and an aversive event (unconditioned stimulus, US), and this alters the neural representation of the CS. Rodent studies have suggested that neurons in auditory cortex shift their tuning curves towards the frequency of a sound associated with threat, implying a stimulus-specific threat representation. However, human research has hinted toward a stimulus-unspecific representation of threat associated with complex visual objects in humans. Here, we seek to elucidate whether the encoding of a threat associated with a CS is linked to specific features. To do so, we defined stimulus sets in each of which CS+ and CS- are distinguished by different features. In two studies, healthy participants underwent a differential delay fear conditioning paradigm in a reinforcement context where the CS+ was probabilistically paired with electrical shock, while the CS- was never paired. In a non-reinforcement context, different sets of sounds were always presented alone (neutral sounds, NS). High-resolution functional MRI was recorded to measure blood-oxygen-level dependent (BOLD) signal associated with the presentation of sounds. Using multivariate pattern analysis (MVPA) of BOLD patterns in superior temporal sulcus (STS), we show that CS identity (CS+ or CS-) can be decoded from neural activity, within each stimulus set, over and above the differences between NS. However, while the identity of the stimulus can be reliably decoded within CS- and NS, responses to the CS+ from the different sets cannot be discriminated. This result is supported by a representational similarity analysis (RSA) revealing formation of a new category defined by threat level. Our results suggest a stimulus-unspecific representation of threat in sensory cortex. This could imply that threat-associated changes in sensory cortex are not simply retunings of receptive fields but crucially involve a new threat encoding.

Disclosures: M. Staib: None. D.R. Bach: None.

Poster

503. Cognition and Anxiety: Human Studies

Location: Hall A
**Abstract:** Background: Aberrant cortical-striatal-thalamic circuitry is a fundamental pathophysiologic mechanism in obsessive-compulsive disorder (OCD; Arnold et al., 2009). OC symptoms occur when an aberrant positive feedback loop develops in the reciprocally excitatory thalamic neuronal interchanges (Modell, 1989). Evidence from resting state studies suggest reduced functional connectivity within cortical-thalamic circuits, but basic cognitive challenges appear to induce task-driven hyper-modulation by regions such as the dorsal anterior cingulate (Diwadkar et al., 2015). Here we assessed task-active modulation by the thalamus of cortical targets in a large group of youth with OCD and controls. Methods: fMRI data were collected in 27 typical controls (age: 12-21, mean = 17.1; 18 males) and 31 OCD youth (age: 13-22, mean = 19.2; 12 males). Subjects underwent fMRI (3T Siemens Verio) using an established verbal working memory paradigm (0-back, 1-back; 30 s epochs, interspersed with 20 s rest epochs). fMRI data were analyzed in SPM8 using typical methods. Psychophysiological interaction was employed to assess contextual modulation by the thalamus of cortical targets (Friston et al., 1997). Time series from the thalamus ($p<.05$, effects of interest) were convolved with the contrast of interest (1back > 0back). The resultant first level maps representing the modulatory effect, were submitted to second level random-effects analysis to identify between group-differences. Results: Relative to typical controls, OCD were characterized by significantly increased modulation of the basal ganglia (Putamen, $t=2.15$, $x=21$, $y=12$, $z=6$), and dorsal prefrontal cortex ($t=2.63$, $x=22$, $y=33$, $z=30$). Conclusion: Our results complement previous studies by suggesting that dysfunctional inputs from the thalamus may exacerbate frontal-striatal circuit dysfunction in OCD. Exaggerated intra-network modulation within the cortical-striatal-thalamic circuit may impede dynamic transitions in network function that are needed to sustain adaptive behavior. Our ongoing network-based assessments of fMRI signals are designed to experimentally address this question.

Title: Racial differences in the emotional response to aversive threat

Authors: *N. G. HARNETT*¹, J. C. LADNIER¹, M. D. WHEELock¹, K. H. WOOD¹, M. A. SCHUSTER² ³, M. N. ELLIOT⁴, S. TORTOLERO⁵, S. MRUG¹, D. C. KNIGHT¹; ¹Psychology, Univ. of Alabama At Birmingham, Birmingham, AL; ²Pediatrics, Harvard Med. Sch., Boston, MA; ³Pediatrics, Boston Children's Hosp., Bostom, MA; ⁴RAND, Santa Monica, CA; ⁵Hlth. Sci. Ctr., Univ. of Texas, Houston, TX

Abstract: Caucasian-American individuals tend to experience internalizing disorders, such as anxiety and depression, at a higher rate than African-American individuals. However, the neural mechanisms mediating these differences are not clear. Anxiety and depression appear to be mediated in part by a network that includes the prefrontal cortex (PFC) and amygdala, wherein altered network activity in these brain regions leads to emotion dysregulation. Pavlovian fear conditioning is often used to investigate neural processes that mediate emotional learning, expression, and regulation. Recent work has demonstrated that the unconditioned response (UCR) elicited by an innately aversive stimulus (unconditioned stimulus; UCS) is diminished when preceded by a warning cue (conditioned stimulus; CS) compared to presentation of the UCS alone (Dunsmoor et al., 2008; Knight et al., 2011; Wood et al., 2012). However, relatively little research has investigated racial differences in the brain regions that mediate conditioned diminution of the UCR. Understanding these differences may provide insight into racial differences in emotion processes related to susceptibility to anxiety and depression. The current study investigated differences in brain (i.e., BOLD fMRI) and behavioral (i.e., SCR and EMG) responses to predictable and unpredictable threat between Caucasian and African-American individuals. We observed diminished brain (e.g., dorsomedial PFC (dmPFC), dorsolateral PFC (dlPFC), inferior parietal lobule (IPL), posterior cingulate cortex, and insula) and behavioral (e.g., SCR) responses to predictable compared to unpredictable threat, consistent with prior conditioned UCR diminution research (Dunsmoor et al., 2008; Wood et al., 2012). However, African-American participants demonstrated diminished threat-elicted responses (independent
of threat predictability) in the dmPFC, dlPFC, ventromedial PFC, IPL, and amygdala compared to Caucasian-American participants. Thus, both groups display conditioned UCR diminution, but African-American participants showed a diminished response to both predictable and unpredictable threat. Follow-up analyses investigated the relationship between the neural response to threat and violence exposure, social support, socioeconomic status, and anxiety. Our findings suggest that socioeconomic status, social support, and anxiety may partially mediate the observed racial differences in the neural activity within these brain regions. The present findings suggest individual differences in socioeconomic status, social support, and anxiety may, in part, account for racial differences in emotional function.


Poster

503. Cognition and Anxiety: Human Studies

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 503.04/K3

Topic: F.03. Motivation and Emotion

Title: Deconstructing white matter connectivity of human amygdala and thalamus subdivisions in vivo

Authors: *A. ABIVARDI, D. R. BACH; Psychiatrische Universitätsklinik Zürich, Zurich, Switzerland

Abstract: Objective: Subcortical amygdalo-thalamic circuits have been suggested to play an important role in early processing of sensory information. In particular, an involvement of a direct pulvinar-amygdala pathway, and more recently an interaction between amygdala and the paraventricular thalamus, has been implicated in processing visual threat information. Such connections have been revealed in non-human animals by semi-quantitative and qualitative tracing methods. However evidence concerning direct subcortical connectivity in humans is still sparse and non-systematic. Here we sought to investigate structural amygdalo-thalamic connections on a subnucleus level by means of probabilistic tractography. Methods: Amygdala parcellation into deep and superficial nucleus groups was implemented in 10 datasets from the human connectome database using a previously established protocol (Bach et al. 2011). This approach is based on voxel-to-voxel connectivity with two distinct cortical areas and uses k-means clustering. Connectivity-based segmentation of the thalamus was based on voxel-to-
region connectivity with frontal, parietal, occipital, temporal, motor and somatosensory cortex. Probabilistic tractography was then performed between these individual amygdala and thalamus segmentations excluding cortical tracts. Results: Among amygdalo-thalamic connections, both amygdala subdivisions showed highest probability of connecting with the temporal parcellation of the thalamus. This corresponds to the dorsal part of the pulvinar and partially to the paraventricular thalamus. Intermediate probabilities of connection, in descending order, were found for the occipital (middle pulvinar regions), parietal (anterior pulvinar) and frontal (MD, VA, VLa, anterior complex) thalamic parcellations. Motor (VLp) and somatosensory (LP, VPL) segments showed low connectivity with the amygdala. Stronger connections with the anterior pulvinar were observed in the deep amygdala nucleus group as well as marginally higher connectivity with the temporal thalamic segmentation for the superficial amygdala. Conclusion: Substantial connectivity between the amygdala and subdivisions of the pulvinar could be shown. Furthermore we found evidence of subcortical connections from the amygdala to paraventricular nuclei. Deep and superficial amygdala nuclei proved to have similar connectivity profiles to the thalamus. Our results consolidate and expand existing evidence of direct anatomical interaction between amygdala and the thalamus in humans and may be used as a guide for functional and pathophysiological neuroimaging studies.

Disclosures: A. Abivardi: None. D.R. Bach: None.

Poster
503. Cognition and Anxiety: Human Studies

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 503.05/K4

Topic: F.03. Motivation and Emotion

Support: Fred B. Snite Foundation

Title: Meta-analysis of sex difference in human amygdala volume

Authors: D. MARWHA, M. HALARI, *L. S. ELIOT;
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Abstract: Statistically-speaking, men and women differ in various measures of social-emotional behavior, including face perception, emotion recognition, fearfulness, and aggression. Each of these behaviors engages the amygdala, a limbic structure that has been proposed to differ both structurally and functionally between males and females. The amygdala has also been found to be reduced in volume in certain CNS disorders that show differential prevalence between sexes,
including borderline personality disorder, PTSD, and unmedicated unipolar depression. We therefore set out to test whether the amygdala is sexually dimorphic, using meta-analyses of amygdala volumes from MRI studies of matched healthy male and female groups of all ages. Using four search strategies, we read 872 unique studies to identify 45 total MRI studies from which we could extract effect sizes for the sex difference in amygdala volume. Among these reports, 35 studies reported raw or uncorrected amygdala volumes from 71 matched samples of males and females, and 13 studies reported data from 29 matched samples in which amygdala volumes were corrected for individual differences in intracranial volume (ICV) or total brain volume (TBV). All data were converted to Hedges g values and pooled effect sizes were calculated using a random-effects model. Each dataset was further meta-regressed against study year and average participant age. We found that uncorrected amygdala volume is about 10% larger in males, with pooled sex difference effect sizes of 1.044 for left amygdala (k=28), 0.880 for right amygdala (k=29), and 0.774 for bilateral amygdala (k=14) volumes (all p values < 0.001). This difference is comparable to measures of both ICV (g=1.108, p<.001, 11.6% larger in males, k=10) and TBV (g=1.268, p<0.001, 10.0% larger in males, k=6) reported in subsets of the same studies, suggesting the sex difference in amygdala volume is a product of larger brain size in males. Among the studies reporting amygdala volumes that were corrected for ICV or TBV, effect sizes were small and not statistically significant, ranging from g=0.257 for bilateral volumes (p=0.131, k=5,) to g=0.230 for the left amygdala (p=0.105, k=12) and g=0.136 for the right amygdala (p=0.380, k=12). These values correspond to about 5% larger corrected left amygdala volume, and less than 0.1% larger corrected right amygdala volume, in males compared to females. In summary, males of all ages exhibit larger amygdala volume than females, but adjusting for individual variation in TBV or ICV statistically eliminates this difference, especially in the right amygdala.


Poster

503. Cognition and Anxiety: Human Studies

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 503.06/K5


Support: NIH 5R01NS038493-15

Neurosurgery Pain Research Institute at the Johns Hopkins University

Title: Contextual fear conditioning in humans using painful laser
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Abstract: Contextual fear conditioning has been used in human neuroimaging studies to reveal the underlying neural mechanisms of learning and memory. During conditioning the unconditioned stimulus (US) is repeatedly paired with conditioned stimulus (CS+) in a particular environmental context (CX+). After repeated pairing, prior to US onset, an elevated skin conductance response (SCR) can be observed and is considered an indication of learning the CS-US association. To date, the functional neuroanatomy underlying the production of SCR is still largely undetermined. Traditional neuroimaging and non-invasive electrophysiological techniques do not provide sufficient spatial-temporal resolutions for studying the neuronal mechanisms underlying the changes of SCR during learning. Earlier human studies of fear conditioning often used highly annoying but not painful electric shocks as US. Less studies incorporated painful stimulus modality as US, and thus the role of US modality in such a conditioning paradigm remains unclear. In the proposed study, we aim to test the hypothesis that elevated SCRs are related to the neuronal mechanisms in the brain, and can be observed during contextual fear conditioning in humans when painful laser is used as US and repeatedly paired with CS+ in CX+. In a group of 9 healthy subjects (2 females; age 23-58 years), we used a 3D display for CSs and CXs presentations and recorded SCR during the entire experiment. The painful laser level (i.e. US) was adjusted to produce a level of 5/10 on the pain rating scale. The conditioning phase consisted of 20 CS+ and 20 CS− trials, all presented within CX+. The entire trial was 21s, and the US was delivered 3s following CS+ offset in 80% of the trials. The extinction phase consisted of 20 trials in total, 10 CS+ and 10 CS− trials, all presented within CX-. Our results showed that the SCR was significantly larger comparing CS+ versus CS- during acquisition (p=0.006, Wilcoxon signed-rank test). The significant SCR differences between CS+ and CS- vanished at the end of extinction (p > 0.05). The presented results provide the initial evidence for the applicability of painful laser during the contextual fear conditioning. Our future work is to compare the neural basis of SCR between nonpainful electric shock and painful laser using high-resolution electrophysiological recordings in patients who are admitted in the epilepsy monitoring units following intracranial depth electrode implantations, and such a study may potentially advance our understanding of the brain mechanisms underlying mental conditions such as PTSD, depression and anxiety.


Poster

503. Cognition and Anxiety: Human Studies
Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 503.07/K6

Topic: F.03. Motivation and Emotion

Support: Wellcome Trust grant 095939

Wellcome Trust grant 098362

Wellcome Trust grant 097490

Title: Biases guiding preference between painful sequences

Authors: *J. S. WINSTON¹, C. NORD¹, K. OHRNBERGER¹, Y.-C. TSENG¹, R. B. RUTLEDGE¹, G. REES¹, I. VLAEV², R. J. DOLAN¹;
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Abstract: When asked to report on hedonic experiences that extend over time, humans and other animals show reliable biases. Two specific heuristics that have been identified as disproportionately affecting self-reports are (i) the hedonic “peak” of the experience (the best or worst part) and (ii) how it ends; these features have been shown to impact judgments about experiences of durations between 1 minute and several years. In a series of experiments on healthy human volunteers, we explored whether such biases extend to short-lasting (6-12s) sequences of cutaneous pain, delivered by electrical stimulation. We found that judgments about painful experiences were influenced by simple heuristics, despite the short timescale. Specifically, evaluation of the overall experience was influenced more by the intensity of stimulus onset than other stimulus components. Intensity at the end of the sequence also exerted an independent bias on evaluation. The relative influence of onset and offset were strongly influenced by the introduction of a concurrent working memory task. Under working memory load, the influence of the stimulus amplitude at onset was diminished while the influence of the end of the sequence was enhanced. Overall, the findings support the idea that mnemonic limitations bias judgments of aversive experiences, even when these unfold over short timescales.


Poster

503. Cognition and Anxiety: Human Studies
Title: Temporal integration of social and emotional cues in social anxiety disorder

Authors: *S. Dubal*, N. George¹, A. Pelissolo², V. Krieger³;
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Abstract: Social anxiety disorder (SAD) is a chronic psychiatric disorder characterized by fear and avoidance of actual or anticipated social situations. The core of the disorder lays in dysfunctional beliefs concerning oneself or the way one is supposed to behave in public that triggers a feeling of being threatened by social situations. According to psychological models, SAD may result from both cognitive factors such as irrational thoughts and an attentional bias towards negative, threatening information. Exploring how SAD patients process face and expressivity from faces is crucial in understanding the pathophysiology of SAD. Emotional face processing bias may explain not only the onset of SAD, but also the persistence of SAD in the clinical population. This study investigated the time course of emotional face perception in SAD patients using EEG. Thirteen SAD outpatients and thirteen control subjects were presented with emotional faces during EEG recording. A block design was used to explore the processing of angry versus neutral faces on the one hand, and happy versus neutral faces on the other. The EEG response pattern of SAD patients was altered relative to control subjects at all processing stages, in response to both angry, happy and neutral faces. In the earliest stages of face processing, around 100 ms after stimulus, activity in the visual cortex was higher in the SAD group than in the control group, whatever the emotional expression presented. This result is compatible with a hypervigilance toward faces, irrespective of its expression during stages of attentional orientation. On the other hand, around 150 ms after stimulus, during face identity processing stage, SAD patients had a decreased activity in the right hemisphere in response to neutral, angry and happy faces as well. At later stages of information processing, around 500 ms after stimulus, the SAD group failed to show modulation of cortical activity by emotion, though the control group did. SAD patients display face processing abnormalities across the entire time range, from perceptual coding to more integrative stages of face and emotional processing. A hypervigilance to faces is then followed by a lowered coding of the structure of faces. Later still, cortical activity is decreased in the SAD group, with less sensitivity to the affective dimension of faces. This last result should be considered in the context of reduced processing of faces.

Disclosures: S. Dubal: None. N. George: None. A. Pelissolo: None. V. Krieger: None.
Title: The tipping point: anterior cingulate and gating of human approach-avoidance decision making

Authors: *M. W. SCHLUND*¹, A. T. BREWER², S. MAGEE¹, D. M. RICHMAN³, S. DYMOND⁴;  
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Abstract: A wide variety of neurophysiological investigations highlight a central role for the anterior cingulate in arbitrating conflict. In approach-avoidance (AA) decision-making, conflict arises when there is a strong competition between available reward and prevailing threat, making cost-benefit analyses difficult. In this functional neuroimaging investigation, an AA conflict task was employed to examine whether the anterior cingulate simply responds to the presence of conflict (binary response) or tracks reward-threat differences (parametric response) in a way consistent with reward-threat arbitration and gating of AA decision making. Thirty healthy adults completed an AA task where monetary reward was fixed ($0.10) but threat ($1 loss) intensity was varied probabilistically (0.0 to 1.0) across trials. Each trial offered a choice to approach a reward in the presence of a CS+ intensity level displayed on a ‘threat meter’ or to avoid. The meter had ten CS+ threat intensities, where low levels were safe (CS-) and higher levels were paired with a greater probability of aversive US delivery. Approach produced the reward and a probabilistic US. In contrast, avoidance reduced threat to a safe CS- level and prevented US delivery. The parametric increase in CS+ threat intensity eventually led to a conflict and a switch from approach to avoidance (the AA transition threshold). Behavioral results showed increasing CS+ threat produced significant increases in ratings of fear, US expectancy, decision times and revealed the AA transition threshold. Imaging results showed increasing CS+ threat was associated with an inverted U-shaped response in pregenual and dorsal anterior cingulate and inferior frontal cortex and a U shaped response in ventromedial prefrontal cortex. For both response profiles, peak activation occurred at a lower threat level than the actual AA transition threshold and well below the uncertainty threshold (where $p$ of loss was .50). Our findings highlighting a parametric response in the anterior cingulate suggests a role in tracking relative...
differences between threat and reward information in a way consistent with reward-threat arbitration and gating of AA decision making in humans.


Poster

503. Cognition and Anxiety: Human Studies

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 503.10/K9

Topic: F.03. Motivation and Emotion

Support: Action for M.E. (UK Charity)

Barts Charity Grant 470/1700

Title: Use of a control task battery to control for pharmacological fMRI investigation of pain responses

Authors: *L. DEMETRIOU*¹, M. B. WALL¹, E. CONSTANTINOU², M. ANTONIADES², J. HOWARD¹, P. D. WHITE², E. A. RABINER¹, J. BOURKE²;
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Abstract: The interpretation of fMRI BOLD response relies on a cascade of cellular events, assuming its relationship with cerebral vasculature is intact; ‘neurovascular coupling’ (NVC). phMRI involves measuring BOLD response combined with a pharmacological agent to measure further neurochemical responses. The use of drugs may disrupt NVC and result in over/underestimations of effects. Separate placebo scans enable robust comparisons of drug effect but do not account for global effects specific to the drug used, independent of the task of interest. A region specific approach allows the use of a control task that activates regions unrelated to the task of interest. The absence of observable effects to the control task in placebo/drug scans suggests that region-specific alterations cannot be accounted for by global vascular effects. We present pilot data examining the efficacy of a brief task battery in assessing for NVC effects in a phMRI study of dopamine and opioid responses to pain in healthy controls and chronic pain patients. Seven controls (mean age 36) and five patients (mean age 39) were recruited. BOLD response was measured during 3 separate fMRI scans paired to oral placebo (always on first scan), naltrexone (NT - 50mg) and amisulpride (AMI - 400mg), separated by
A brief (5 minute) task battery was developed, which included auditory, calculation, language, motor and visual stimuli presented in an event-related design with 3s trials and 10 conditions presented in a pseudo-random order. A pressure-pain paradigm was then used to examine BOLD responses to pain under drug conditions. Data was available for 12 placebo, 11 NT and 10 AMI scans. Both drug conditions (relative to placebo) produced clearly dissociable group effects on pain responses in key brain regions; generally increasing pain responses in the control group and decreasing them in the patients. However, a region of interest analysis on the control task battery data revealed only an effect in the AMI condition on the auditory component. This effect was present in both participant groups; there was no interaction with the between-subjects group variable. NT also had no effect on responses to the control task battery. The effect of AMI on the auditory task alone may represent a combination of the scanner environment and the fact that dopamine differentially modulates neural activity in auditory processing. The use of such control tasks is simple and improves the validity of phMRI studies, potentially filling the gap between fMRI and positron emission tomography (PET) methodologies on the frontier of behavioral research.

**Disclosures:** L. Demetriou: A. Employment/Salary (full or part-time); Imanova Ltd. M.B. Wall: A. Employment/Salary (full or part-time); Imanova Ltd. E. Constantinou: None. M. Antoniades: None. J. Howard: A. Employment/Salary (full or part-time); Imanova Ltd. P.D. White: None. E.A. Rabiner: A. Employment/Salary (full or part-time); Imanova Ltd. J. Bourke: None.

**Poster**

**503. Cognition and Anxiety: Human Studies**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 503.11/K10

**Topic:** F.03. Motivation and Emotion

**Title:** Human fear conditioning follows ideal bayesian learning

**Authors:** *A. TZOVARA*¹, D. R. BACH²;
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**Abstract:** Using environmental cues to predict threat is crucial for survival, particularly in dynamic environments, where associative learning is required. Models of aversive learning propose different mechanisms, based on signed or unsigned prediction errors (Rescorla-Wagner - RW- and Pearce-Hall -PH- models, respectively), or on integrating prior expectations with
incoming evidence (Bayesian Learning models -BL-). These different models try to explain the same behaviour but give rise to different and often opposing predictions in terms of how instructive signals are computed and passed in neural networks. Here, we capitalised on Bayesian model selection to arbitrate between different learning models based on activity of the parasympathetic nervous system. In a delay fear conditioning experiment, healthy volunteers were presented with two neutral conditioned stimuli (CS+/−), one of which was paired with an aversive unconditioned stimulus (US) in 50% of trials. Threat prediction on each trial was inferred from phasic sympathetic responses, estimated from skin conductance responses in a dynamic causal model. We implemented three different families of learning models, following the RW, PH and BL rules, each including models with different levels of complexity (i.e. number of free parameters), and one family which assumed that no learning took place (NL). Model evidence was quantified as Bayesian information criterion (BIC), calculated from residual error. We used fixed and random effects analysis (FFX/RFX) for a formal model comparison. Results showed strong evidence of learning, indexed by significantly higher Bayes factors (FFX) and exceedance probabilities (RFX) for learning vs. non-learning model families. Within the learning models, model evidence was clearly in favor of BL compared to RW and PH. Among two implementations of the BL model, significantly stronger evidence was found for the less complex one, assuming uninformative beta priors for CS+/CS-. Our results suggest that ideal Bayesian learning best explains sympathetic conditioned responses during delay fear conditioning. This result crucially constrains the search for a neural implementation of delay fear conditioning in terms of aversive instructive pathways. Further studies will investigate whether other forms of conditioning, such as trace conditioning also conform to ideal Bayesian learning.

Disclosures: A. Tzovara: None. D.R. Bach: None.
Abstract: The “need to belong” is an important evolutionary motivation for humans. Social rejection has been associated with a host of psychological and physiological morbidities including major depressive disorder (MDD). Once MDD develops, abnormal responses to social rejection or acceptance may reinforce symptoms and contribute to poor treatment outcomes. The present study compared neural responses to social rejection and acceptance in patients with MDD to a matched group of healthy controls. Participants were 18 medication-free female patients with current MDD (30 ± 10.8 years) and 19 healthy female controls (31.4 ± 12.0 years). MDD patients were diagnosed by structured clinical interview, with an average score of 17 on the 17-item Hamilton Depression Rating Scale, and were free of antidepressant medication for at least six months at the time of the study. Subjects rated online profiles of preferred-sex individuals with whom they were most likely to form a close relationship. During functional magnetic resonance imaging (fMRI) they were given feedback that they were liked or not liked by their highest-rated profiles. A priori regions of interest (ROIs) included areas within the “social pain-matrix” including the dorsal anterior cingulate cortex (dACC) and the anterior insula (AI) for rejection, and reward regions including the nucleus accumbens (NAcc) and amygdala. In both healthy controls and depressed subjects, acceptance trials corresponded to increased activation in the NAcc. Furthermore, rejection trials increased activity in the “pain-matrix” (dACC and AI) in depressed subjects, but not in healthy controls. These results indicate that there are systematic differences in how depressed individuals process social feedback.


Poster
503. Cognition and Anxiety: Human Studies

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 503.13/K12

Topic: F.03. Motivation and Emotion

Support: ESRC First grant

Title: Simultaneous appetitive and aversive classical conditioning: an fMRI study
Authors: *D. TALMI*1, R. HOSKIN2;
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Abstract: Being able to attenuate fear by using information about the long-term reward associated with a scary prospect is a key aspect of emotional intelligence. Our goal here was to elucidate the neural correlates of this emotion regulation skill. Emotion regulation has often been studied in the laboratory with re-appraisal paradigm, where participants are asked to re-appraise stimuli that have been paired with aversive outcomes in a positive manner. For example, participants may be asked to use the color of a fear-conditioned stimulus to trigger an image of a relaxing scene. However, because the re-appraisal paradigm is open-ended, we know little about how re-appraisal instructions were implemented at a mechanistic level. Here we used joint appetitive-aversive classical conditioning paradigm to study this mechanism. As in previous research, participants learned to fear inherently neutral stimuli by taking part in a classical conditioning paradigm with a 2 (threat anticipation: yes/no) x 2 (reward anticipation: yes/no) design. Participants learned about the value of four different stimuli during the course of the experiment. Two images predicted physical pain, and two predicted safety. One of the stimuli that predicted pain and one that predicted safety also predicted that monetary reward will be added to participants’ account, and paid at the end of the experiment. The other two predicted that no reward will be delivered. The value of the reward was titrated individually so that it was equivalent to the (aversive) value of the painful stimulus, using the Becker-DeGroot-Marschak method. We found that the interaction between threat and reward anticipation was expressed in the inferior frontal cortex, where activation for the conflict-ridden condition (pain and reward anticipation) was stronger than the activation for the pain only or the reward only conditions.

Disclosures: D. Talmi: None. R. Hoskin: None.

Poster

503. Cognition and Anxiety: Human Studies

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 503.14/K13


Support: JSPS KAKENHI Grant Number 26245678

Title: A genome-wide association study of the long-term clinical response to SSRI or SSRI with antipsychotics in obsessive-compulsive disorder in the Japanese population
Authors: *H. UMEHARA*¹, S. NUMATA¹, A. TAJIMA²,³, A. NISHI¹, I. IMOTO², S. SUMITANI¹, T. OHMORI¹;
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Abstract: Object: Obsessive-compulsive disorder (OCD) is a neuropsychiatric disorder with a prevalence of approximately 2%. Selective serotonin reuptake inhibitors (SSRI) are well-established first-line pharmacological treatments for OCD, and antipsychotics are used as an augmentation strategy for SSRI in SSRI-resistant patients with OCD. The purpose of the present study is to identify genetic variants and pathways which are associated with the long-term clinical response to SSRI or SSRI with antipsychotics in OCD. Methods: We performed a genome-wide association study in 93 OCD patients. The patients were divided into three groups according to pharmacological response, as evaluated by the Yale Brown Obsessive-Compulsive Scale (Y-BOCS): group A, responders to a SSRI (n=54); group B, responders to a SSRI with an atypical antipsychotic (n=22); and group C, non-responders to a SSRI with an atypical antipsychotic (n=17). Patients who showed a >35% decrease on the Y-BOCS after treatments were considered responders. These patients were evaluated at baseline and at the end of 6- to 36-month treatment (mean follow-up duration: 13.1 ± 8.1 months). To evaluate the effect of each SNP on the clinical response (SSRI response (group A vs. group B plus C) or the response to a SSRI with antipsychotics (group B vs. group C)), logistic regression analysis was performed using the PLINK software with adjustment for covariates sex, age, onset age and Y-BOCS baseline score. A pathway-based analysis was conducted by Improved Gene Set Enrichment Analysis for Genome-wide Association Study (i-GSEA4GWAS). Results: We didn’t reveal any genetic variants associated with clinical responses to SSRI or SSRI with an atypical antipsychotic at genome-wide significance. However, we identified 8 enriched pathways in the SSRI treatment response and 5 enriched pathways in the treatment response to SSRI with an antipsychotic medication (FDR < 0.05). Conclusion: Our results may provide the clues of the mechanisms of treatment response in OCD.


Poster

503. Cognition and Anxiety: Human Studies

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 503.15/K14
Topic: F.03. Motivation and Emotion

Support: VA ORD CSR&D I01 CX000771

Title: Computer-based “avatar” to assess avoidant behaviors in participants with symptoms of posttraumatic stress disorder (PTSD)

Authors: *C. E. MYERS*1,2, Y. T. EBANKS-WILLIAMS1, M. L. RADELL1, K. D. BECK1,2, M. W. GILBERTSON3;
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Abstract: Symptoms of posttraumatic stress disorder (PTSD) include cognitive and behavioral avoidance of reminders of a traumatic event. PTSD symptoms can be assessed through clinical interview as well as via self-report questionnaires, such as the PTSD Checklist (PCL), which queries for frequency of specific PTSD-related symptoms. The PCL is well-validated and highly predictive of clinical PTSD, but like all self-report questionnaires it can be subject to demand characteristics as well as vulnerable to unawareness of deficit. It would therefore be useful to have a behavioral tool for the assessment of avoidant behavior, which generates scores correlated with PTSD symptomatology. A viable approach may be virtual environments, in which participants guide avatars through a series of on-screen events that simulate real-world situations. We have been developing such a tool, where participants select avatars to represent themselves and then guide the avatars through several simulated scenarios, such as attending a party or helping out at a volunteering event. At each choice point, participants are asked what the avatar would do next, and can select from predefined response options corresponding to avoidant, non-avoidant, or neutral behaviors. In an initial sample of putatively healthy young adults (college students), scores on the avatar task were strongly predictive of the personality trait of behavioral inhibition (BI), a tendency to withdraw from or avoid novel situations that is a risk factor for PTSD and anxiety disorders. The relationship between performance and BI held regardless of participant gender or prior experience with computer games. Here, we considered a sample of veterans and community civilian controls (mean age 52 years), assessed for PTSD symptoms via the PCL. Over half of the sample met symptom criteria for PTSD based on PCL. Results show a strong correlation between task scores and PCL scores; additionally, subjects reporting diagnosed PTSD scored significantly higher on the task than those reporting no PTSD or anxiety disorders. Importantly, the relation between PTSD symptoms and task performance held even though the task did not specifically invoke trauma-related imagery or events, but rather simply scored how the avatar behaved in various social situations. These results suggest that virtual environments may hold promise as alternative formats for observing, rather than merely querying, avoidant behaviors related to PTSD symptomatology, and also add to a growing literature documenting that avoidant behaviors in those with PTSD symptoms may represent a general cognitive bias not limited to trauma-related situations.

Poster
503. Cognition and Anxiety: Human Studies

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 503.16/K15

Topic: F.03. Motivation and Emotion

Support: Clinical Science Research and Development Service of the VA Office of Research and Development I01CX000771

Title: Gender differences in avoidance behavior in individuals with post-traumatic stress disorder (PTSD) symptoms

Authors: *M. L. RADELL1, J. SHEYNIN2,3, K. D. BECK1,4, K. C. H. PANG1,4, C. E. MYERS1,4;
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Abstract: Women are more likely to be diagnosed with post-traumatic stress disorder (PTSD), and exhibit differences in symptom presentation and course, compared to men. Exaggerated avoidance is a prominent feature of PTSD and potential differences in how men and women learn to avoid aversive events could, in part, underlie the observed gender differences in vulnerability. Therefore, understanding gender differences in PTSD might provide the opportunity to tailor treatment to the individual and improve clinical outcomes. Previously, we examined avoidance learning in putatively healthy young adults using a computer-based task and found that women acquired avoidance behavior to a greater degree than men. Here, we examined avoidance learning in veterans and civilians (mean age 53 years) with self-reported PTSD symptoms in a similar task. Participants could learn to shoot an enemy spaceship to earn points and hide their own spaceship from an enemy to avoid losing points. On each trial, a warning signal predicted subsequent point loss caused by an enemy firing at the participant’s spaceship. Subjects could move their spaceship into safe areas to completely avoid losing points, or to escape from enemy fire, terminating ongoing point loss. While there was a conflict between approach and avoidance in our previous study, here, we limited the opportunity to earn points to a safe intertrial interval. PTSD symptoms were also quantified via the PTSD Checklist (PCL), a well-validated self-report questionnaire predictive of clinical PTSD. Subjects were split into low
PTSD symptom (low PTSS) and high PTSD symptom (high PTSS) groups based on PCL scores (<50 or 50+, respectively). While most subjects learned to escape, a subset did not, and this included more “low PTSS” women. Among subjects who did learn to escape, “low PTSS” women escaped less than “high PTSS” women, and less than men regardless of PTSS. Finally, in contrast to our study in young adults, the results so far show that “low PTSS” women also tended to avoid less. Age differences between the studies could account for this discrepancy. Since approach-avoidance conflict was removed from the current study, associations with gender may also depend on the presence of competing responses, i.e. where the ability to obtain reward precludes avoidance. Finally, the results suggest that women with more PTSD symptoms are, as expected, more susceptible to avoidance than women with fewer symptoms, even in non-trauma-related situations. It remains unknown if this is due to preexisting differences in associative learning that confer vulnerability to PTSD in women who will later experience trauma or results from developing the disorder.


**Poster**

**503. Cognition and Anxiety: Human Studies**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 503.17/K16

**Topic:** F.03. Motivation and Emotion

**Support:** Stress and Motivated Behavior Institute

University of Northern Colorado

**Title:** Differential effects of us alone trials: pre-exposures, but not interpolated trials, disrupt acquisition of conditioned eyeblinks in anxiety vulnerable individuals

**Authors:** *T. ALLEN*¹², D. P. MILLER¹², R. J. SERVATIUS⁴²; ¹Sch. Psych Sci., Univ. Northern Colorado, Greeley, CO; ²Stress and Motivated Behavior Inst., Syracuse, NY; ³Neurosci., Carthage Col., Kenosha, WI; ⁴Syracuse Veterans Affairs Med. Ctr., Syracuse, NY

**Abstract:** Behavioral inhibition (BI), defined as a temperamental tendency to withdraw from or avoid novel social and non-social situations, enhances acquisition of conditioned eyeblink responses (CRs). Holloway et al. (2012) reported enhanced proactive interference of eyeblink
conditioning following US alone or CS alone pre-exposures in individuals with high trait anxiety. More recently, acquisition of CRs did not differ between a partial reinforcement protocol with 50% US alone (corneal air puff) trials as compared to 100% CS-US (tone-air puff) trials (Allen et al., 2014). This was surprising given the reduction in paired trials in the 50% US alone. Previously, Kimble et al. (1955) tested a protocol with an interpolated block of 20 US alone trials during CS-US training and reported that CRs did not differ from those receiving 100% paired trials. We sought to extend the work with US alone training both when pre-exposed and interpolated into training sessions include behavioral inhibition. Undergraduates completed personality inventories including the Adult Measure of Behavioural Inhibition (AMBI). All participants received 60 acquisition trials. Acquisition consisted of either 100% CS-US training, 20 CS-US trials followed by 20 US alone trials, followed by 20 more CS-US trials or 30 US alone pre-exposures followed by 30 CS-US trials. BI individuals exhibited more CRs than non-inhibited individuals in the 100% CS-US training protocol and the Kimble et al., protocol. The interpolated US alone trials did not disrupt CRs which replicated the findings of Kimble et al. The US alone pre-exposures disrupted acquisition of eyeblink CRs which replicated the findings of Holloway et al. In addition, US alone pre-exposures eliminated the enhanced acquisition normally observed in BI individuals. The effects of unpredicted aversive stimuli on learning and anxiety will be discussed in the light of uncertainty, learned helplessness, and cue salience.


Poster

503. Cognition and Anxiety: Human Studies

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 503.18/K17

Topic: F.03. Motivation and Emotion

Support: Biomedical Laboratory Research and Department of Veterans Affairs Office of Research & Development I01BX000218

Biomedical Laboratory Research and Department of Veterans Affairs Office of Research & Development I01BX000132

NIH RO1-NS44373

Title: Assessing behavioral flexibility in anxiety vulnerable rats using a novel aversive strategy shifting task
Abstract: Behavioral flexibility is the ability to alter learned behaviors in response to unexpected changes in the environment, and is dependent on the ventromedial prefrontal cortex (vmPFC). Several psychiatric disorders, including anxiety disorders, are associated with vmPFC dysfunction and exhibit a lack of behavioral flexibility. Strategy shifting tasks used to assess behavioral flexibility have traditionally employed appetitive motivation, whereas tasks used to assess anxiety employ aversive motivation. In this study we sought to determine whether behavioral flexibility in anxiety vulnerable rats differs between appetitive and aversive motivated strategy shifts. Anxiety vulnerable Wistar Kyoto (WKY) rats naturally exhibit inflexible behavior in the form of perseverative avoidance. In the first experiment, WKY and Sprague Dawley (SD) rats were tested in an appetitive maze-based strategy shifting task. In this task, rats were presented with two stimulus domains (color and texture) with only one domain predictive of reward. WKY rats acquired the initial discrimination faster than SD rats. Both strains expressed similar performance during reversal learning (intra-domain change), but, WKY rats acquired extra-domain shifts faster than SD rats. In a second experiment, WKY and SD rats will be tested in a novel, aversive operant-based strategy shifting task. In this task, rats will be presented with two stimulus domains (lever-location or light-location) with only one domain capable of terminating shock. We predict that WKY rats will again acquired initial discrimination faster than SD rats and both strains will express similarities in reversal learning. In contrast to the appetitive strategy shifts, we predict that WKY rats will have more difficulty in aversive strategy shift compared to SD rats. These results would provide a novel way to assess the negative bias underlying anxiety disorders.

Authors: *J. SHEYNIN*¹,², S. A. GEORGE¹,², R. GONZALEZ³, I. LIBERZON¹,², J. L. ABELSON²;
¹Veterans Affairs Ann Arbor Healthcare Syst., Ann Arbor, MI; ²Dept. of Psychiatry, ³Dept. of Psychology, Univ. of Michigan, Ann Arbor, MI

Abstract: Anxiety disorders interfere with individuals' ability to perform daily tasks, and are often associated with specific decision-making biases. For instance, loss aversion is a well-documented human tendency - defined as a willingness to sacrifice attainable gains in order to avoid equivalent potential losses. Data suggest that loss aversion is increased in anxiety, but studies have rarely examined clinical populations across the full range of anxiety disorders. Here, we investigated the influence of anxiety disorders on variables that shape decision-making under risk, using the Cumulative Prospect Theory framework (Tversky & Kahneman; 1992). Eighty-seven patients recruited from an academic Anxiety Disorders Clinic and 20 control subjects were given a battery of self-report clinical and personality measures and completed a computerized decision-making task. The task required a series of decisions in which subjects chose preferred gambles based on varying probabilities of winning or losing varying amounts of points. We employed the two key elements of the Prospect Theory: (1) Utility function, which models the diminishing sensitivity for increasing gains/losses, as well as the loss-aversion phenomenon; (2) Probability function, which models the tendency to over-weight small probabilities and under-weight moderate and high probabilities. Parameters were estimated for each subject to generate a close fit of subject’s performance on each trial of the task. Preliminary findings suggest that anxious individuals, across multiple diagnoses, have altered processing of the value of gains and losses, consistent with evidence of similar disruptions in post-traumatic stress disorder. This investigation will shed light on anxiety-related decision making biases involving approach and avoidance tendencies, and examine differences across various anxiety disorders. Such biases may contribute to sub-optimal decision making and maladaptive behavior in patients with anxiety. (JS and SAG have equally contributed to this work.)


Poster

503. Cognition and Anxiety: Human Studies

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 503.20/K19

Support: Gjensidige Foundation

Title: Functional changes in brain activity in response to recent traumatic experiences: a trauma specific fMRI study

Authors: *A. S. NILSEN*¹, I. BLIX², S. LEKNES², T. HEIR¹; ¹Norwegian Ctr. For Violence and Traumatic Stress, Oslo, Norway; ²Intervention Ctr., Oslo Univ. Hosp., Oslo, Norway

Abstract: Exposure to traumatic events such as assault, injury, combat, accidents and death, can develop into Post-Traumatic Stress Disorder (PTSD). The past 20 years an increasing number of studies have focused on the neurobiological mechanisms of PTSD, however, short term neurobiological changes of trauma exposure, pre-PTSD diagnosis, are not well understood. We employed fMRI to investigate functional changes in response to trauma specific, threatening and neutral visual stimuli (images selected from the IAPS database). Twenty three trauma exposed participants (females=5, age=40.1±12.5) who were recently (within 3 weeks) involved in serious traffic accidents, performed a standardized picture task including road traffic accidents (trauma specific), violence (threatening) and neutral pictures. The trauma exposed group was matched on gender, age and education with 21 healthy controls (females=7, age=36.9±9.2). We analyzed functional blood oxygenation response between conditions and groups while controlling for symptom severity as measured by the PTSD checklist (PCL). Based on the existing literature of fMRI studies of negative emotional stimuli in trauma exposed participants with PTSD, we hypothesized that trauma exposed participants without PTSD would show higher activation of amygdala and reduced activation of medial prefrontal cortex, during viewing of trauma specific and/or threatening images relative to neutral images. A secondary hypothesis was that activation patterns in these regions would reflect symptom severity, as measured by the PCL. Contrary to the main hypothesis, preliminary results indicate no significant hyperactivation in amygdala or hypoactivation in medial prefrontal cortex when watching trauma specific or threatening stimuli in trauma exposed participants, as compared to the control group. Furthermore, the activation patterns were not significantly modulated by symptom severity. Compared to controls, participants who had recently been involved in a traffic accident did show significantly higher activation in the visual system along the dorsal and ventral visual streams, including bilateral superior colliculi, fusiform cortex and parahippocampal gyrus during viewing of trauma specific images. These results are consistent with an increased perceptual sensitivity to trauma specific material in this group at ~3 weeks after the traumatic event. We discuss these findings as potential early indications of risk of (or protection against) development of PTSD.

Disclosures: A.S. Nilsen: None. I. Blix: None. S. Leknes: None. T. Heir: None.

Poster

504. Mood Disorders Animal Models I
Title: Time-dependent changes induced by acute stress in function and architecture of excitatory synapses in prefrontal and frontal cortex

Authors: *M. POPOLI*, L. MUSAZZI, P. TORNESE, N. SALA, G. TRECCANI, C. BAZZINI, G. WEGENER, J. NYENGAARD, N. NAVA; 1Univ. Degli Studi Di Milano, Milano, Italy; 2Aarhus Univ. - Dept. of Clin. Med., Aarhus, Denmark; 3Aarhus Univ. - Ctr. for Stochastic Geometry and Advanced Bioimaging, Aarhus, Denmark

Abstract: Stressful life events impact on brain and bodily function and represent major risk factors for stress-related neuropsychiatric disorders. The response to stressful events can promote adaptive plasticity and improved cognition, when the physiological stress response is efficiently activated and inactivated in due time, or maladaptive and harmful effects, when the response is overused or dysregulated. In turn, the outcome of a maladaptive stress response can be associated with the triggering of brain, systemic and metabolic disorders. Chronic stress has been shown to induce reduction of density of synapses and dendrites in prefrontal and frontal cortex (PFC/FC), with concomitant impairments in neuronal activity and cognitive functions. Instead, the early and rapid effects of acute stress on synaptic function and plasticity are often opposite, with enhancement of glutamate release/transmission, increased number of spines and synapses, enhancement of synaptic strength. However, the delayed effects of acute stress have not been investigated, although this could give crucial information on the time-dependent changes in the brain stress response. We have previously characterized the synaptic effects of acute footshock (FS)-stress, which induces enhancement of glutamate release/transmission in PFC/FC, due to the increase of the readily releasable pool (RRP), in turn mediated by rapid non-genomic corticosterone action at synapses (Mol. Psy., 19:433–443, 2014). Here we have analyzed the effects of acute FS-stress in the PFC/FC of rats at different times after completion of the stress protocol. We found that acute stress induced early and sustained increase of RRP over time in excitatory perforated synapses, while the number of non-perforated and axo-spinous synapses...
was increased (without changes in vesicle pools). The total number of synaptic spines was increased up to 24 h, while apical dendrites showed decreased density 2 weeks after acute stress (with no significant changes at earlier times). In behavioral tests for working memory, FS-stress improved performance 2 h after stress and impaired it after 24 h. Changes in glutamate release, RRP, number of synapses and spines are blocked or attenuated by prior chronic treatment with the antidepressant desipramine. The different glutamatergic modifications in functional and morphological plasticity suggest a bi-phasic process, during which the stress response in PFC/FC may turn from early increased excitatory activation into its opposite. The identification of these points and the players involved in the switch are crucial for the understanding of the dynamics of stress-related pathology.

patients with psychiatric disorders, including schizophrenia and bipolar disorder. Overexpression of glucocorticoid receptor (GR) in the forebrain during early life and throughout the lifetime causes increased depression-like and/or anxiety-like behaviors in mice, suggesting that mice overexpressing GR (GRov mice) represent a potential animal model for mood disorders, such as depression and anxiety disorder. Overexpression of GR has been shown to cause an aging-like neuroendocrine phenotype and mild cognitive dysfunction in young mice, raising the possibility that GRov mice may have an "hyper-matured" brain status. In this study, to assess whether cellular maturity of brain cells in the mice is altered, we compared genome-wide gene expression in the DG of GRov mice with those of the corresponding regions in normally developing brains of wild-type (WT) mice by using a bioinformatics tool, NextBio. The DG of GRov mice exhibited an opposite expression pattern of immaturity and maturity marker genes when compared to the developing DG in WT mice. We further tried to find microarray data sets that have statistically significant similarity in their gene expression patterns to those in GRov mice among more than 56,900 publicly available microarray data sets. Hippocampal gene expression patterns in the glutamate dehydrogenase 1 (Glud1) transgenic (Tg) mice, which shows aging-related biochemical and physiological phenotypes, and in the mice treated with PF-04447943, a selective phosphodiesterase-9 inhibitor, were significantly similar to those in the DG of GRov mice. These mice also displayed contrasting gene expression patterns in the hippocampus to those in developing DG in WT mice. These observations indicate that maturation status of the hippocampal cells might be altered toward ‘hyper-maturity’ in GRov mice, Glud1 Tg mice, and PF-04447943-treated mice, suggesting that cellular hyper-maturation is a common phenomenon shared by a certain type of state in the brain induced by some genetic or pharmacological manipulations.

Abstract: Depression is caused by a combination of genetic and environmental factors. In addition, it has been proposed that epigenetic mechanisms may also contribute to the risk for depression. Recent evidence suggests that the offspring of males who are susceptible to chronic social defeat stress (CSDS) display increased depressive- and anxiety-like behaviors but only after the fathers are exposed to stress. The mechanisms by which such parental experience influences stress susceptibility of their offspring are poorly understood. We tested the hypothesis that changes in sperm cells during CSDS are responsible for encoding increased susceptibility to stress in F1 and F2 offspring. Male mice were exposed to 10 days of CSDS and subjected to social interaction testing to assess paternal phenotype. Males identified as resilient or susceptible to CSDS, as well as control F0 males, were allowed to mate 30 days after the stress, to allow the sperm exposed to the stress to mature. One week following natural mating, those same F0 males were euthanized and their sperm was collected for artificial insemination and snap frozen for epigenetic analysis. At ~P60, 1 male and 1 female in each litter was exposed to subthreshold unpredictable stress and depression- and anxiety-like behaviors were assessed. Each animal was compared to an unstressed littermate of the same sex to control for litter effects. Additionally, phenotypes of offspring produced via natural mating and artificial insemination were compared between litters sired by the same father to investigate if the phenotype was passed via the paternal germ cells. Preliminary evidence suggests that F1 offspring of defeated fathers produced by natural mating or by artificial insemination display altered phenotypes, with opposite effects seen in males and females. Paternal stress augmented stress susceptibility of F1 males, while reducing it in F1 females. These data suggest that there are changes in sperm during CSDS that encode altered depressive- and anxiety-like phenotypes in their offspring. Transgenerational inheritance is currently being investigated by assessing similar outcomes in the F2 generation, and epigenetic changes are being investigated in the sperm of the F0 fathers to identify the molecular mechanisms of this paternal transmission.

**Title:** Dissociative depression-related behavioral effects of cholinergic signaling in the ventral tegmental area versus the nucleus accumbens

**Authors:** *K. M. SMALL, E. J. NUNES, N. A. ADDY*; Dept. of Psychiatry, Yale Univ., New Haven, CT

**Abstract:** Preclinical studies report that cholinergic signaling in both the ventral tegmental area (VTA) and the nucleus accumbens (NAc) is involved in regulating depression-related behaviors. Cholinergic receptors on multiple neuronal populations in the VTA and NAc can robustly alter dopaminergic activity that is known to mediate susceptibility to stress. However, it remains unclear whether cholinergic mechanisms in the VTA and NAc similarly or distinctly mediate behavioral responses to stress and anxiety that are implicated in depression. Thus, the goal of the present study was to determine whether an increase in cholinergic tone in the VTA or the NAc would similarly modulate various depression-related behaviors. Adult Sprague-Dawley male rats, were surgically implanted with a bilateral cannula into the VTA or NAc. After a recovery period of one week, we examined whether pharmacological manipulation of VTA and NAc cholinergic tone altered responding in the forced swim test (FST) and the sucrose preference test. We found that VTA administration of the acetylcholinesterase inhibitor, physostigmine (0, 1, or 2 µg/side), increased immobility time in the forced swim test and decreased sucrose preference in a dose-dependent manner. In contrast, physostigmine (0, 1, or 2 µg/side) administered into the NAc dose-dependently decreased immobility time in the forced swim test but had no effect on sucrose preference. The physostigmine effects in the VTA suggest susceptibility to stress while the effects in the NAc are suggestive of an antidepressant-like response. These findings provide new insight into the role of cholinergic activity in the mesolimbic circuitry in depression-related behavior.

**Disclosures:** K.M. Small: None. E.J. Nunes: None. N.A. Addy: None.

**Poster**

**504. Mood Disorders Animal Models I**
**Abstract:** The Canadian Mental Health Association estimates that major depression affects approximately 8% of Canadians at some point in their lives, and up to 30% of those affected do not respond to traditional therapeutics. Low doses of ketamine, a N-methyl-D-aspartate (NMDA) receptor antagonist, have been found to alleviate depressive symptoms more rapidly than conventional anti-depressants particularly in treatment-resistant patients. Recent research suggests that the anti-depressant effect of ketamine is specific to NMDA receptors containing the GluN2B subunit. In this study, we ran an antidepressant screening of the novel GluN2B-specific antagonist Ro 8-4304 hydrochloride to determine if the drug could reduce immobility time in the Porsolt Forced Swim Test (FST); a traditional preclinical screening test of potential anti-depressant medications. 20 male Balb/c mice were divided into 4 groups and were given intraperitoneal injections of saline, 5mg/kg, 10mg/kg, and 15mg/kg of Ro 8-4304, respectively. All mice were tested one hour post-injection using the FST. The 15mg/kg group exhibited a statistically significant trend towards reduced immobility in the FST. While low dose ketamine treatment has been found to produce an increase in brain-derived neurotrophic factor (BDNF) in the hippocampus, our study did not find significant increases in BDNF in any of the treatment groups. These findings are sufficient to suggest that a dose of 15mg/kg of Ro 8-4304 may have antidepressant effects, and therefore should be studied more extensively using a chronic mild stress paradigm to determine its full treatment potential and to elucidate its mechanisms of action.

**Disclosures:** N. Prowse: None. K. Farmer: None. S. Hayley: None.

**Poster**

504. Mood Disorders Animal Models I

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM
Title: Mechanism of lithium-induced recovery of memory and emotional impairment in DGKbeta KO mice

Authors: *W. OKIMOTO*1, M. ISHISAKA2, H. NAKANISHI3, S. UEDA1, M. YAMANOUE1, T. Sasaki3, H. Harai2, Y. Shirai1;
1Kobe Univ., Kobe, Japan; 2Gifu Pharmaceut. Univ., Gifu, Japan; 3Akita Univ., Akita, Japan

Abstract: Diacylglycerol kinase (DGK) converts diacylglycerol to phosphatidic acid. To date, 10 mammalian subtypes of DGK have been identified. Among them, DGKβ is enriched in neuron. We have developed DGKβ knockout (KO) mice to investigate function of DGKβ in nervous system, and revealed that they have emotional and memory disorder with decrease of spine density. Interestingly, both disorders were rescued by 10 days lithium treatment [1,2]. However the mechanism is still unknown. Therefore, to elucidate the mechanism, we measured spine density after the lithium treatment, and found that, spine density in KO mice was recovered with lithium, indicating that spine density is a key for both impairment and recovery of memory and emotion of the KO mice. And now, we are investigating changes in lipid metabolism in neurons from the KO mice before and after the lithium treatment.

Abstract: Dorsal raphe nucleus (DRN) is a hindbrain structure located below the aqueduct. It consists of 2/3 of serotonergic neurons, and is important to regulating emotion-related behaviors. It has been proved that DRN receive dense inputs from interpeduncular nucleus (IPN). However, the circuit between IPN and DRN is poorly investigated. Here we injected Cre-dependent rabies virus in DRN of GAD2-Cre and ePet 1-Cre mice respectively in order to trace the monosynaptic inputs of DRN GABAergic and serotonergic neurons retrogradely. Results showed that IPN neurons were strongly labeled in ePet 1-Cre mice, in contrary, a handful of IPN neurons were labeled in GAD2-Cre mice. Immunohistochemistry results showed that the neurons projecting from IPN to DRN labeled by rabies virus were mostly CaMKII positive. Taken together, these results showed that IPN CaMKII positive neurons project to DRN, and target serotonergic neurons preferentially.

Disclosures: Y. Li: None. X. Li: None.

Poster

504. Mood Disorders Animal Models I

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 504.08/K27


Title: Pre-existing variability in functional connectivity predicts behavioral response to acute social defeat in mice

Authors: *Y. GROSSMAN, D. DUMITRIU;
Icahn Sch. of Med. At Mount Sinai, New York, NY

Abstract: Depression is a neuropsychological disorder that affects millions of people. There are currently no predictors for susceptibility to depression and a large proportion of afflicted individuals are resistant to available treatments. The ability to predict selective psychosocial vulnerability and resilience to stress holds great promise in preventing this debilitating disorder. Social defeat (SD) is a highly validated mouse model of depression. In order to elucidate the pre-existing neurocircuitry involved in the establishment of socially avoidant (susceptible) and resilient behavioral phenotypes, we developed an acute model of SD (ASD) that closely correlates with chronic SD. Using this behavioral model in conjunction with cFos immunohistochemistry, whole slice imaging and semi-automated detection of fluorescent puncta,
we quantified the cellular activation in 49 brain regions one hour after ASD in behaviorally characterized mice (18 control, 15 resilient, 21 susceptible). Post-hoc region-to-region and global network analyses revealed that resilient animals exhibit higher correlative inter- but not intra-network activity compared to susceptible animals. Overall, our data suggests that there is pre-existing variability in functional connectivity contributing to divergent behavioral phenotype following ASD.


Poster

504. Mood Disorders Animal Models I

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 504.09/K28


Title: Behavioral and physiological effects of oxytocin treatment in a rat model of post-traumatic stress disorder

Authors: *D. P. DABERKOW, M. D. RENICKER, N. G. CYSEWSKI, S. M. PALMER, D. V. NAKONECHNYY, A. J. KEEF; Biol., Eastern Washington Univ., Cheney, WA

Abstract: Post-traumatic stress disorder (PTSD) is characterized by anxiety, hyperarousal, flashback memories and avoidance of reminders of the traumatic event. Dysregulated fear and stress responses put an individual at an increased risk for developing PTSD. Oxytocin has been suggested to reduce certain PTSD-like behaviors and resulting cognitive functional impairments. The objective of this study was to investigate the effects of oxytocin treatment on electric shock-induced PTSD-like symptoms. METHODS: Oxytocin treatments were accomplished by intranasal administration of oxytocin (0.1 µg/kg) or natural oxytocin release as a result of massage-like stroking. PTSD-like symptoms were induced via inescapable foot shock in a fear conditioning chamber (26 cm x 26 cm). Adult, male Sprague Dawley rats (n=24) were divided into four groups (n=6, per group): control (no shock treatment and intranasal administration of saline), PTSD-control (exposed to foot shock and intranasal administration of saline), PTSD-stroke (exposed to foot shock and administered massage-like stroking to evoke natural oxytocin release), and PTSD-oxytocin (exposed to foot shock and intranasal administration of oxytocin). One week post fear conditioning, exposed rats and controls (not exposed to foot shock) were evaluated behaviorally and physiologically using a range of tests including: freezing when re-exposed to the fear conditioning chamber, anxiety assessment in elevated zero maze, spatial
learning in Morris water maze, heart rate and hematocrit. RESULTS: Relative to controls, oxytocin treated rats showed significantly less conditioned fear (periods of watchful immobility when re-exposed to the foot shock chamber), significantly less anxiety-related behaviors in the elevated zero maze (time in walled off areas), significant improvement in spatial learning measures in the Morris water maze (time to reach the hidden platform), significantly lower resting heart rate and hematocrit levels. CONCLUSIONS: The results of this study suggest that oxytocin treatment reduces anxiety, improves learning and physiological measures related to stress. Therefore, these data support further investigation of oxytocin administration in the prevention of PTSD induction.


Poster

504. Mood Disorders Animal Models I

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 504.10/K29


Support: Colgate University

Title: Comparison of behaviors under Light versus Dark conditions in the neoclomipramine rodent model of Obsessive Compulsive Disorder (OCD)

Authors: L. S. LAIKS1,2, A. M. BARNES1, E. C. GRONSETH1, A. M. SOLIN1, L. BURKE1, L. J. KASPARSON1, C. MATTHIJSSEN1, A. SCALZO1, *D. S. KREISS1,3; 1Psychology and Neurosci. Dept, Colgate Univ., Hamilton, NY; 2Neurosci., Texas A & M Inst. for Neurosci., College Station, TX; 3Psychology Dept, Neurosci. Program, Ithaca Col., Ithaca, NY

Abstract: Obsessive Compulsive Disorder (OCD) is characterized by persistent, anxiety producing thoughts accompanied by overwhelming urges to perform repetitive, ritualistic behaviors. Current pharmacological treatments for OCD are only effective in 40-60% of patients and have an 8-10 week delayed onset. The current study provides further evaluation of a new animal model of OCD based upon neonatal exposure to an uptake inhibitor of both serotonin and norepinephrine, clomipramine. Prior studies conducted by Anderson et al., 2010 and at Colgate (SFN & FUN abstracts, 2013 and 2014) have demonstrated that the neoclomipramine model has both face and predictive validity for behaviors in the Hole-Board, Marble Arena, and Elevated
Plus Maze. A major limitation of prior studies of the neoclomipramine model is that the behaviors were assessed in the Light, a factor known to increase anxiety in nocturnal species. For the first time, head dips, holes poked, marbles buried, marbles checked, and marbles carried were measured in the Dark - in both control and experimental neoclomipramine rats. Male Sprague-Dawley rats were administered either clomipramine (15 mg/kg, "neoClom," n=23) or saline ("neoSal", n=28) twice daily from postnatal Day 9 through Day 16. Behaviors were first assessed at Day 68-85 and again at Day 172-184 under standard Light conditions. After a 2-week adjustment period to a reverse light-dark cycle, the rats were assessed under Dark conditions (using red lamps). In accordance with prior studies conducted in the Light, neoClom rats buried, checked, and carried more marbles than the neoSal rats both at Day 68-85 and at Day 172-184. When these behaviors were assessed in the Dark at Day 206-213, neoSal animals exhibited a REDUCTION of pokes/hole, repeated pokes, marbles carried, and marbles carried - and, unexpectedly an ENCHANCEMENT of marbles buried. In contrast, expression of behaviors of neoClom rats in the Dark was NOT different from the neoClom’s behaviors in the Light. In the Dark, neoClom rats continued to exhibit higher values of marbles buried, checked, and carried as compared to their control counterparts. These results strengthen the face validity of hole-poke, marble checking and marble carrying behaviors in the neoclomipramine model, yet challenge the proposition that marble burying is a valid measure of “OCD-like” behavior. Furthermore, this study contributes to the growing evidence that behavioral observations in animal models of anxiety-related disorders exhibit higher face validity if conducted under Dark conditions.


Poster

504. Mood Disorders Animal Models I

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 504.11/K30


Support: NIMH Grant MH099851

Title: Behavioral effects of enhancing GABAergic neurotransmission through disinhibition of somatostatin-positive interneurons in mice
Abstract: Clinical evidence suggests that Major Depressive Disorder (MDD) is associated with reduced concentrations of the inhibitory neurotransmitter γ-aminobutyric acid (GABA) in certain brain regions\(^1,3\), reduced expression of GABA type A (GABA\(_A\))\(^4\) receptors and reduced expression of the inhibitory neuron marker somatostatin (SST)\(^2\). Consistent with a causal role of GABAergic deficit in the etiology of MDD, reduction of GABAergic neurotransmission in mice through global heterozygous deletion the γ2 subunit of the GABA\(_A\) receptor results in an anxious-depressive-like phenotype that is normalized by chronic but not acute treatment with the antidepressant desipramine\(^5\). These data suggested that modest GABAergic deficits may be causal for depressive disorders and, conversely, that chronic antidepressants act over time to enhance GABAergic transmission. Accordingly, we hypothesized that increasing the excitability of somatostatin (SST)-positive GABAergic interneurons would result in an antidepressive phenotype. To generate mice with increased excitability of SST\(^+\) interneurons we inactivated the γ2 subunit gene (gabrg2) selectively in SST\(^+\) interneurons (SST-Cre x γ2\(^{f/f}\)). To facilitate recordings from SST\(^+\) interneurons we further crossed these mice with a Rosa26-YFP Cre-reporter (SST-Cre x γ2\(^{f/f}\) x Rosa26-YFP). Consistent with increased excitability of SST\(^+\) neurons, the input resistance and number of action potentials observed following current injection into SST\(^+\) cells of SST-Cre x γ2\(^{f/f}\) x Rosa26-YFP neurons was increased compared to SST-Cre x γ2\(^{f/+}\) x Rosa26-YFP controls. Conversely, analyses of pyramidal cells in L2/3 of the cortex and CA1 region of the hippocampus by voltage clamp revealed an increased frequency and amplitude of IPSCs, as predicted. Behaviorally, SST-Cre x γ2\(^{f/+}\) mice showed an anxiolytic and antidepressive phenotype characterized by reduced time spent in the open arms of an elevated plus maze, reduced latency to feed in the novelty suppressed feeding test, decreased immobility in the forced swim test and fewer escape failures in the learned helplessness test. Finally, SST-Cre x γ2\(^{f/+}\) mice showed brain region-specific biochemical changes indicative of antidepressant drug action, including decreased phosphorylation of eEF2. Collectively, the results suggest that enhancing GABAergic inhibition of forebrain principal cells by increasing the excitability of SST\(^+\) GABAergic interneurons confers an anxiolytic and antidepressive phenotype.


Poster

504. Mood Disorders Animal Models I

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM
Title: Adult neurogenesis in the hippocampus is responsible for the transition from depressive to manic behavior

Authors: *S. KIM, J.-M. LEE, D. GEUM;
Korea Univ. Col. of Med., Seoul, Korea, Republic of

Abstract: Recently, it has been reported that adult neurogenesis in the hippocampus is involved in not only learning and memory but also mood regulation. In the present study, we generated Nestin-CreER;Rosa26-FloxedlacZ-DTa (Nestin-DTa) mice whose nestin-positive adult neural stem cells (aNSCs) were ablated by tamoxifen treatment, and we evaluated the function of hippocampal neurogenesis on the depressive and manic behavior. Five-consecutive daily injections of tamoxifen (5mg/injection) ablated aNSCs, and resulted in the decrement of nestin positive cells and BrdU-positive proliferating cells to 50% in Nestin-DTa mice. Four weeks after tamoxifen injections, when new born neurons matured and were integrated into neural circuits, behavior tests such as learned safety, open field test (OFT), elevated plus maze (EPM) and forced swimming test (FST) were performed. The Nestin-DTa mice could not learn safety learning, which clearly shows the ablation of hippocampal neurogenesis. The Nestin-DTa mice spent less time in the center of the open field during OFT, and stayed longer in closed arms in EPM than those of control mice, which demonstrated the higher anxiety level. The Nestin-DTa mouse also showed depressive behavior such that they exhibited more immobilization time during FST. However, eight weeks after tamoxifen injections, the higher anxiety level shown in Nestin-DTa mice was recovered to that of control level. More interestingly, 8 weeks after tamoxifen injections, Nestin-DTa mice showed manic like behavior during FST. We also analyzed the population of aNSCs, proliferating cells, immature neuroblasts and mature neurons in Nestin-DTa mice with immunohistochemistry. Four weeks after tamoxifen injections, BrdU positive newly born mature neurons that were incorporated into the circuit were decreased. However, proliferating cells (PCNA+) and immature neuroblasts (Dcx+) were repopulated in spite of the loss of sox2 positive stem cells. Eight weeks after tamoxifen injections, proliferating cells and immature neuroblasts were re-populated in Nestin-DTa mice. Interestingly, however, newly born mature neurons were over-populated than control mice. Behavior tests in concordance with histological analyses suggest that the population of newly born mature neurons in the hippocampus that may incorporate into the neural circuit is important for the mood regulation such as depressive and manic behavior. Key words: Hippocampus, Neurogenesis, Depressive behavior, Manic behavior.

Disclosures: S. Kim: None. J. Lee: None. D. Geum: None.
Title: A depressive-like brain state caused by GABAergic deficits involves a homeostatic adaptation of glutamatergic synapses that is normalized by ketamine

Authors: Z. REN1, H. PRIBIAG2, M. SHOREY2, T. FUCHS3, D. STELLWAGEN4, *B. LUSCHER3;

Abstract: Mechanisms underlying major depressive disorder (MDD) are increasingly recognized to involve deficits in GABAergic and glutamatergic synaptic transmission. To elucidate the relationship between these phenotypes we here made use of GABAergic receptor (GABAAR) γ2 subunit heterozygous (γ2+/-) mice, which have previously been established as model with construct, face and predictive validity for MDD. Cortical cultures derived from γ2+/- embryos showed reduced cell surface expression of NMDA and AMPARs along with the synaptic cell adhesion protein neuroligin 1 (NL1), as well as reduced density of glutamatergic synapses. All these phenotypes were restored to wild-type (WT) levels by 3-6 h treatment of γ2+/- cultures with 10 µM ketamine. Cell surface NMDARs and NL1 but not AMPARs were also reduced in vivo as shown for hippocampus and medial prefrontal cortex of γ2+/- mice. Functionally, glutamatergic inputs to CA1 pyramidal cells of γ2+/- mice were reduced mainly due to functional impairment of temporoammonic-CA1 synapses; however, Schaffer collateral-CA1 synapses were also affected. A single subanesthetic dose of ketamine given to γ2+/- mice resulted in enduring normalization of NMDAR cell surface expression and function of glutamatergic synapses in slices harvested and analyzed 24h post treatment of mice. Moreover, ketamine had more modest effects in WT than γ2+/- mice. The amplitude of miniature inhibitory postsynaptic currents was reduced in CA1 neurons of γ2+/- compared to WT mice and remained functionally constrained in slices of ketamine treated γ2+/- mice. Collectively these results newly
define depressive-like brain states as GABAergic deficit-induced homeostatic adaptations of glutamatergic synapses that are reversible enduringly by ketamine.

**Disclosures:** Z. Ren: None. H. Pribiag: None. M. Shorey: None. T. Fuchs: None. D. Stellwagen: None. B. Luscher: None.

**Poster**

**504. Mood Disorders Animal Models I**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program/#/Poster#:** 504.14/K33

**Topic:** C.16. Cognitive, Emotional, and Behavioral State Disorders

**Support:** UNH Department of Psychology

**Title:** Voluntary wheel running alters intermittent swim stress-induced ultrasonic vocalizations

**Authors:** *R. C. Drugan, I. Stribling, N. P. Stafford*

Dept Psychology, Univ. New Hampshire, Durham, NH

**Abstract:** Exercise has been shown to impart resilience in animal stress paradigms. The typical exercise protocol involves 6-8 weeks of voluntary running in the home cage. We have recently reported that certain rats exposed to intermittent swim stress (ISS) emit ultrasonic vocalizations (USVs). The rats that emit USVs during ISS later show stress resilience when tested on both anxiety and depression measures. In the current study, we sought to determine if rats given the opportunity to engage in voluntary exercise for 6 weeks would show enhanced USVs during ISS and show the expected resilience in a subsequent forced swim test (FST). Preliminary results indicate that certain rats given the opportunity to exercise emit more USVs than the sedentary controls and show commensurate resilience in the FST.

**Disclosures:** R.C. Drugan: None. I. Stribling: None. N.P. Stafford: None.
**Program#/Poster#:** 504.15/K34

**Topic:** C.16. Cognitive, Emotional, and Behavioral State Disorders

**Support:** 2011-0028772 to D.K

**Title:** Left cortical activity modulates stress effects on social behavior

**Authors:** *J. HONG*¹, S. CHAE¹, E. LEE¹, Y.-G. PARK¹, K. KANG², Y. KIM¹, D. KIM¹; ¹KAIST, Taejon-City, Korea, Republic of; ²Dankook Univ., Chungnam, Korea, Republic of; ³Rockefeller Univ., New York, NY

**Abstract:** In response to chronic stress, some individuals develop maladaptive symptoms while others retain normal behavior. The functional hemispheric asymmetry of medial prefrontal cortex (mPFC) is known to be implicated in control of stress responses. Despite the potential importance of mPFC, the contribution of each hemisphere of mPFC in mediating stress resilience remains unclear. To elucidate the specific role of each hemispheres, we investigated the neural activity and gene expression profiles of mPFC in social defeat stress model. In the left mPFC, mice expressing social avoidance showed depressed neural activity, while resilient mice showed normal firing rates. The neural activity stimulation of left mPFC optogenically leads to social behavior change. The same photomodulation has no effect on the right mPFC. From the analysis of microarray data in each mPFC hemispheres, we discovered that the genetic changes in the left cortices determines the adaptive behavior upon social defeat stress. Evidenced together with the neural activity, we conclude that it is the activity of left mPFC plays critical role in behavioral expression of stressed mice.

**Disclosures:** J. Hong: None. S. Chae: None. E. Lee: None. Y. Park: None. K. Kang: None. Y. Kim: None. D. Kim: None.

**Poster**

**504. Mood Disorders Animal Models I**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 504.16/K35

**Topic:** C.16. Cognitive, Emotional, and Behavioral State Disorders

**Title:** Behavioral differences due to recent de novo mutations among C57BL/6 and C57BL/10 mouse substrains
Authors: *C. ST. PIERRE, N. M. GONZALES, A. A. PALMER;
Univ. of Chicago, Chicago, IL

Abstract: C57BL/6 (B6) mice are the most widely used inbred strains in biomedical research. Several B6 substrains exist that have been maintained as isolated breeding populations for several decades. These B6 substrains are known to exhibit numerous of behavioral differences. In addition, C57BL/10 (B10) mice have also given rise to a number of substrains, which are most commonly used for immunological rather than behavioral studies. We subjected a panel of 13 different B6 and B10 substrains to a battery of behavioral tests relevant to addiction and psychiatric disorders. The panel included eight C57BL/6 strains (C57BL/6J, C57BL/6NJ, C57BL/6N-TyrC/BrdCrlCrl, C57BL/6N-Crl, C57BL/6ByJ, and C57BL/6NHsd) and five C57BL/10 strains (C57BL/10J, C57BL/10ScCr, C57BL/10ScSnJ, C57BL/10SnJ, and C57BL/10ScNHsd). We found significant differences in the behavior of the substrains in the open field test, locomotor response to cocaine, fear conditioning, prepulse inhibition, and Porsolt forced swim test. Our results show a divergence of behavioral performance within these substrains, suggesting that recent de novo mutations that alter disease-relevant CNS pathways have accumulated since the strains were separated. We are currently re-sequencing individuals from each substrain in an effort to identify causal polymorphisms.

Disclosures: C. St. Pierre: None. N.M. Gonzales: None. A.A. Palmer: None.

Poster

504. Mood Disorders Animal Models I

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 504.17/K36


Support: INPRF NC123240.1

Title: Effect of amygdaloid kindling and administration of fluoxetine in the rat forced swim test

Inst. Nacional De Psiquiatría Ramón De La Fuen, Mexico, Mexico

Abstract: Depression represents one of the most common comorbidities of epilepsy. However, causes and mechanisms of depression associated to epilepsy remain poorly understood. It has
been suggested that shared biological factors might both cause seizures and lead to affective disturbances. Experimental models have shown that antidepressants, such as fluoxetine (FLX) affect excitability in short time scales which may lead to changes in epilepsy severity. Amygdaloid kindling (AK) is a model of epilepsy characterized by sustained increase in seizure susceptibility. Furthermore AK allows temporal control over both seizure generation and severity. Thus, it appears to be particularly suitable for epilepsy-associated depression models that results from neuronal plastic changes. Therefore, the goal of the present study was to examine AK epileptogenesis with behavioral correlates of depression in the rat forced swim test (FST) as well as the effects of FLX on seizure susceptibility and depressive behaviors. Male Wistar rats (280-320 g) were used. Tripolar electrodes were placed in the basolateral nucleus of left temporal lobe amygdala (P: 2.8, L: 5.0, H: 8.5) and both frontal cortices. AK was induced by daily electrical stimulation (1 s train, 1 ms pulses, 60 Hz, 250-500 µA) until AK stage V (tonic-clonic seizure) was achieved for three consecutive days. One hour after last seizure, FST sessions were conducted by placing rats in individual glass cylinders (46 cm height; 20 cm diameter, water at 25-28 °C). An initial FST of 15-min (termed pre-test) was performed and after 24 h, a 5-min test was completed. A 10 mg/kg of fluoxetine hydrochloride (2 ml/kg) dose was administered following a sub-acute schedule, three injections administered between pre-test and test sessions (21 h, 5 h, and 1 h before test session). Rats were assigned to four experimental groups: K-Flx (n=7), in which AK, FST and FLX injections were applied; K-Vh (n=6) AK, FST and vehicle (saline solution 0.9%); Sham-Vh (n=6) FST and vehicle and Control (C) (n=7) solely with FST. Immobility time in FST and seizure susceptibility on test stimulations of post stage V were assessed. We found a decrement in immobility time in the FST on all groups compared with C (p< 0.001), whereas both K-Vh and K-Flx groups exhibited an increase in seizure susceptibility (p< 0.001), nevertheless no difference between groups was observed. Our results suggest that the FLX has no effects on seizure susceptibility, thus the neuronal plastic changes associated with limbic system, particularly with the amygdala, could be interfered by the development of depressive behavior.


Poster

504. Mood Disorders Animal Models I

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 504.18/K37
Cannabinoid receptor 1 blockade in the lateral habenula exerts anxiolytic effects in Sprague Dawley rats

*A. Berger*1, A. M. Williams1, R. J. McLaughlin2;  
1Psychology, 2Integrative Physiol. and Neurosci., Washington State Univ., Pullman, WA

Abstract: Clinical and preclinical research has identified the lateral habenula (LHb) as a key structure regulating motivated and stress-related behaviors, such as anxiety, in part through its strong inhibitory effects on midbrain monoaminergic nuclei. Extensive research has also established the endocannabinoid (eCB) system as an important regulator of stress responsiveness and emotional behavior. Despite this overlap, very little research has evaluated the role that eCBs play in the LHb in the context of anxiety. The goal of this study was to examine the role of the eCB system in the lateral habenula with respect to anxiety-like behavior. Adult male Sprague Dawley rats were surgically implanted with bilateral cannulae aimed at the LHb. Following recovery from surgery, animals were subjected to the elevated plus maze and novelty suppressed feeding paradigms, which are both measures of anxiety-like behavior in rodents. Animals were pretreated with the CB1R inverse agonist rimonabant (0.3 µg/side), a cocktail of baclofen and muscimol (75ng/side), or vehicle infusion. Compared to vehicle infusions, animals receiving rimonabant unexpectedly exhibited a significant increase in open arm exploration in the elevated plus maze, indicative of reduced anxiety, as well as a trend for decreased time to consume a palatable food item in the novelty suppressed feeding paradigm. Animals receiving the baclofen/muscimol cocktail exhibited a non-significant increase in open arm exploration in the elevated plus maze and did not show altered behavior in the novelty suppressed feeding paradigm. These findings are indicative of anxiolytic properties of CB1R antagonism in the LHb and contribute to our understanding of how signaling in the LHb influences anxiety-like behaviors. This data also points to the LHb as the potential site of action for the anxiolytic effects of CB1R blockade.

Disclosures: A. Berger: None. A.M. Williams: None. R.J. McLaughlin: None.
Support: NIMH Grant R21MH099562
NIMH Grant T32MH87004-4
NIMH Grant T32MH096678-02
NIMH Grant F31 MH105217

Title: Sex specific stress regulation of the microRNA transcriptome in mouse nucleus accumbens

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Abstract: Adult women are twice as likely as men to develop major depression, although the precise molecular mechanisms underlying sexual dimorphism in depression susceptibility are unknown. We have developed a stress paradigm--subchronic variable stress (SCVS)--to mimic these sex differences in mice. Female mice subjected to SCVS exhibit depression-like behavior after 6 days of stress exposure, whereas male mice exhibit this behavior only after chronic stress exposure (21 days, but not 6 days, of SCVS). Thus, this model is a valid recapitulation of the sexual dimorphism characteristic of the human population. We profiled sex differences in gene expression and transcriptional regulation in the mouse nucleus accumbens (NAc), an essential structure in the processing of reward and motivation, using next generation mRNA and small RNA sequencing. Both types of sequencing were performed on the same NAc tissue from reproductively intact male and female mice subjected to SCVS. Bioinformatic analysis was used to determine differential mRNA and microRNA (miR) expression patterns, predict miR targets, create miR-gene networks and identify biological pathway enrichment. We find very little overlap in stress-regulated mRNA and miR profiles between male and female mice. miR target, network and pathway analyses revealed evidence for sex differences in regulation of molecular processes related to stress vulnerability in males and females. Our results demonstrate that male and female mice initiate fundamentally different transcriptional responses to stress. These transcriptional profiles correlate with behavioral susceptibility or resilience to stress. Our findings provide insight into the molecular underpinnings of enhanced female susceptibility to stress.


Poster
Title: Underlying molecular circuitry of miR-124-mediated glutamatergic pathway regulation by corticosterone: role in stress-related pathophysiology

Authors: *B. ROY1, R. C. SHELTON1, G. TURECKI2, Y. DWIVEDI1;
1Psychiatry and Behavioral Neurobio., Univ. of Alabama At Birmingham, Birmingham, AL;
2Douglas Mental Hlth. Res. Inst., Montreal, QC, Canada

Abstract: Imbalance in Cortisol- or Corticosterone-mediated delicate regulation of limbic-hypothalamic-pituitary-adrenal axis can induce physiological stress, a critical factor in inducing stress-related disorders, including major depression. Understanding the underlying neuro-molecular circuitry of stress response reveals a complex set of biological mediators involving both genetic and epigenetic candidates in prefrontal cortex, amygdala and hippocampus. In recent years, microRNA (miRNA or miR) has established its potential role as epigenetic master regulator of gene expression in central nervous system by affecting genes involved in neurotransmission and synaptic plasticity. In this study a chronic paradigm of 50 milligram corticosterone (CORT) administration in rat model exhibited increased expression of a brain enriched miR-124 in prefrontal cortex. Underpinning the molecular nature of this miRNA unveiled an intricate switch involving CORT-mediated post transcriptional regulation of a candidate gene Gria4, earlier reported to be a subunit of AMPA receptor family in glutamatergic pathway. The concomitant CORT-mediated downregulation of Gria4 was substantiated by the observation of similar transcriptional repression in prefrontal cortex of depressed subjects. In-vitro target validation models established Gria4 3’UTR as direct target of miR-124 using luciferase and miRNA oligo transfection assays. This observation was further supported by RNA-Induced Silencing Complex (RISC)-mediated immuno enrichment study of endogenous miR-124 seed binding with Gria4 mRNA 3’UTR. Interestingly, our observation of high miR-124 abundance in serum samples of patient population suffering from major depression potentially consolidated the role of miR-124 as a biomarker for MDD and established a strong relationship
with impaired Gria4 expression possibly translating its role in the pathophysiology of depression. As an additional layer of transcriptional regulation on miR-124 expression, the upstream proximal promoter was found to be populated with two CpG islands. Exploring a significant hypomethylation status of the miR-124 promoter under chronic stress correlated well with the observed transcriptional upregulation of miR-124 expression. Altogether the present study exhibits a molecular switching mechanism induced by corticosteron leading towards transcriptional upregulation of miR-124 with a possible disruption in glutamatergic pathway by post transcriptional silencing of Gria4 transcripts, which could potentially be important in the pathophysiology of stress-related disorders.

**Disclosures:** B. Roy: None. R.C. Shelton: None. G. Turecki: None. Y. Dwivedi: None.

**Poster**

504. Mood Disorders Animal Models I

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program/#/Poster#:** 504.21/K40

**Topic:** C.16. Cognitive, Emotional, and Behavioral State Disorders

**Support:** Morrison Trust

**Title:** Differential impact of maternal protein insufficiency on 5-HT1A receptor function in adult offspring: increased risk for affective disorders?

**Authors:** *W. YE*¹, B. J. THOMPSON², J. G. HENSLER³;


**Abstract:** Genetic programming and pharmacological manipulation of serotonin1A (5-HT1A) receptors have been associated with anxiety. Protein insufficiency, without caloric restriction, during pregnancy leads to elevated anxiety in female but not male adult offspring. However, the underlying neural mechanisms are unclear. In rats, we examined 5-HT1A receptor function in adult offspring of mothers that were free-fed standard (20%) or low (10%) protein isocaloric chow during pregnancy, which models protein intake patterns in the US. Importantly, the maternal low protein diet did not affect the body weight of mothers or offspring. Preliminary data indicate that the maternal low protein diet does not alter the number of 5-HTergic cell bodies in the dorsal and median raphe, or 5-HT content or turnover in the forebrain of offspring. At P110-117, 5-HT1A receptor agonist 8-OH-DPAT (0.01-1mg/kg, ip)-induced stereotypic
behaviors were measured. The potency of 8-OH-DPAT to induce flat body posture and lower lip retraction was reduced in female offspring, as indicated by rightward shifts (~3 fold) in the dose-response curves. In littermates, we used quantitative autoradiography to measure the capacity of 5-HT1A receptors to activate G proteins. The maternal low protein diet resulted in a (50%) decrease in 8-OH-DPAT (1µM)-stimulated [35S]GTPγS binding in hippocampus of female offspring, with no change in frontal cortex, suggesting a region-specific down-regulation of postsynaptic 5-HT1A receptor function. 8-OH-DPAT (1µM)-stimulated [35S]GTPγS binding was increased (54%) in dorsal raphe of female offspring, suggesting enhanced inhibition of serotonin neuronal activity. By contrast, no significant change in 5-HT1A receptor function was observed in male offspring. Both in vivo and ex vivo data indicate that maternal protein insufficiency causes profound region-specific and sex-dependent changes in 5-HT1A receptor function. As improperly balanced diets and nutritional insecurity remain considerable problems in developed countries, including the US, these findings have important implications for understanding why some individuals are at greater risk for affective disorders.

**Disclosures:**  W. Ye: None.  B.J. Thompson: None.  J.G. Hensler: None.

**Poster**

**504. Mood Disorders Animal Models I**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:**  504.22/K41

**Topic:** C.16. Cognitive, Emotional, and Behavioral State Disorders

**Support:** National Institute of Neurological Disorders and Stroke (NINDS) Grant R01NS085200 (PI: Nanyin Zhang, PhD)

National Institute of Mental Health (NIMH) Grant R01MH098003 (PI: Nanyin Zhang, PhD)

**Title:** Characterizing rat model of post-traumatic stress disorder using neuroimaging approaches

**Authors:** *P. D. PEREZ, J. RUDDY, Y.-K. YAM, J. KUHN, N. ZHANG;

Biomed. Engin., Pennsylvania State Univ., University Park, PA

**Abstract:** Chronic stress-related disorders can develop when a situation overwhelms the physical and/or emotional resources of the individual. A single life threatening experience, in the event of a posterior maladaptation, can develop into disorders such as post-traumatic stress disorder (PTSD), excessive anxiety and phobias. PTSD is a complex disorder that is poorly...
understood. Furthermore, there is heterogeneity in the response to traumatic stress and its eventual development in a chronic disorder. Most studies in humans have focused on restricted populations that had already been exposed to traumatic stress months or years ago, such as veterans and accident and crime victims. The use of an animal model allows for investigating the development of PTSD before the stress exposure, which is extremely difficult in humans. We use a rat model of PTSD where we expose rats to a single life threatening situation (predator odor) in an inescapable environment. Using a combination of behavior and awake resting-state functional magnetic resonance imaging (rsfMRI) experimental techniques, we can study the effect of single-episode traumatic stress in neuroplasticity at the circuit level. Furthermore, we investigate how single traumatic stress affects fear conditioning and fear extinction learning which are suspected to be staples of chronic stress disorders. We use rsfMRI to study the function of neural circuits critical to anxiety disorders. We complement rsfMRI with behavior techniques to evaluate the efficacy of the stressor and anxiety levels. Our study has multiple goals. We present a longitudinal set of experiments where we can first study the onset of chronic disorder, then its impact in fear conditioning circuits and finally in fear extinction circuits. Furthermore, we observe a segregation of resilient and vulnerable populations. By looking at differences between these two populations at different stages, we investigate the existence of vulnerability markers to PTSD at the circuit level with non-invasive techniques. Understanding and detecting this vulnerability has great importance in prevention and treatment, and it opens the way for later extending this knowledge to human studies.

**Disclosures:** P.D. Perez: None. J. Ruddy: None. Y. Yam: None. J. Kuhn: None. N. Zhang: None.

**Poster 504. Mood Disorders Animal Models I**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 504.23/K42

**Topic:** C.16. Cognitive, Emotional, and Behavioral State Disorders

**Support:** UBACYT 20020130100258BA

PIP CONICET

UBACYT 20020130300033BA

**Title:** Neuronal features involved in depressive disorders are found in CB1receptor knockout mice
Abstract: Endocannabinoid system (CB1 and CB2 receptors, endogenous ligands, and the synthesis and metabolism associated enzymes) plays a fundamental role during development of nervous system. CB1R is expressed mainly in the central nervous system, is located at presynaptic level and is the main responsible for the effects produced by cannabinoids in the brain. It regulates physiological processes such as appetite, memory, pain regulation, humor and learning among others. It plays a key role in the neuromodulation of synaptic activity, neurogenesis and synaptogenesis. It has been proven that the absence of the CB1 receptor, in experimental mice, as well as the use of antagonist drugs to that receptor in humans, generates behavioral states similar to depression, stating its presence and involvement as a key element for the proper functioning of the nervous system. The aim of this work is to analyse the neuronal morphology (cytoskeletal organization in axons and dendrites, and synaptic contact ultrastructure) as well as the serotonergic system in CB1R knockout mice. The changes observed in these parameters are in accord with the alterations observed in depressive disorders. Funding: UBACYT 20020130100258BA (HAB), 20020130300033BA (LC), PIP CONICET 00269

Disclosures: H.A. Brusco: None. D. Soriano: None. F. Conde: None. L. Caltana: None.

Poster

504. Mood Disorders Animal Models I

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 504.24/L1


Support: NIH DA033380

NIH OD018339

NIH OD018340

AFSP SRG-1-135-11

Title: Modeling the role of Akt kinase in resilience to stress

Authors: C. D. WILLOCK¹, N. C. BERDUX¹, *T. F. FRANKE²;
¹Psychiatry, ²Psychiatry, Biochem. and Mol. Pharmacol., NYU Sch. of Med., New York, NY
Abstract: Background: Akt kinase plays a key role in neuronal cell function and survival. Biochemical studies on postmortem brains from suicides define the importance of Akt signaling in mood regulation. Human genetic studies provide evidence that AKT1 mutations are a liability in neurodevelopmental disorders with high incidence of suicide such as schizophrenia. Our project builds on these remarkable findings to examine tractable genetic mouse models of altered Akt signaling that “reverse-translate” changes in human Akt to determine their impact on anxiety- and depression-like behaviors. Methods: To define the physiological involvement of Akt signaling on the processing of emotional stimuli, we used Akt knockout mouse strains to study Akt-dependent behavioral and biochemical phenotypes, and structural neuroplasticity. To address the contribution of Akt in defined brain areas, we employed Cre-dependent recombination to ablate Akt expression in the frontal cortex and mesolimbic system. Genomic and brain area-specific conditional knockout mice were exposed to acute and chronic stress, and assessed for anxiety- and depression-like behaviors and treatment response to antidepressant treatment. Results: Akt-deficient mice differed significantly in the acquisition of fear memories. Consistent with changes in fear memory acquisition, Akt KO mice exhibited decreased synaptic plasticity. Acute stress exposure of Akt KO mice resulted in a transient depression-like phenotype. Chronic exposure to social defeat uncovered a reduced resilience to stress. Ablation of Akt expression specifically in the frontal cortex also decreased resilience of mutant mice when compared to littermate controls. Treatment of chronically-stressed mice with antidepressants reversed the depression-like phenotype in control mice but failed to ameliorate depression-like behaviors in Akt-mutant mice. Discussion: Our results in Akt-mutant mice converge with biochemical and genetic findings of Akt dysfunction in neuropsychiatric patients and confirm a critical requirement for Akt signaling in mood regulation. Decreased Akt function in the frontal cortex of mice increased the susceptibility to chronic stress and occluded reversal of depression-like behaviors by antidepressants. Our data validate the utility of Akt-mutant mice as experimental model to study gene-environment interactions governing the susceptibility to stress and development of depression-like behaviors later on in life. A mechanistic understanding of the role of fronto-cortical Akt signaling in stress resilience will help to develop new diagnostic markers and therapeutic interventions.

Disclosures: C.D. Willock: None. N.C. Berdux: None. T.F. Franke: None.

Poster

504. Mood Disorders Animal Models I

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 504.25/L2
Title: Long-lasting alterations in microglial HMGB1 expression correlates with increased vulnerability to depressive-like behaviors after chronic unpredictable stress

Authors: *T. C. FRANKLIN, E. S. WOHLEB, R. S. DUMAN; Yale Univ. Sch. of Med., New Haven, CT

Abstract: Long-lasting alterations in microglial HMGB1 expression correlates with increased vulnerability to depressive-like behaviors after chronic unpredictable stress. T.C. Franklin; E.S. Wohleb; R.S. Duman Major Depressive Disorder (MDD) is a recurrent mental health illness with more than half of affected patients relapsing after their initial episode. High levels of psychological or environmental stressors are associated with the initial development of MDD and may play a role in recurrent episodes. To better understand these processes it is important to study stress-associated mechanisms that promote depressive-like behavior. Various stress paradigms have been used to model the molecular, cellular and behavioral effects of depression. For instance, several reports support the hypothesis that repeated stress exposure increases neuroinflammation, which contributes to the development and persistence of depressive-like behaviors. In this study, we identified long-lasting microglial changes that corresponded with development and recurrence of MDD using a chronic unpredictable stress (CUS) model in rodents. Following CUS exposure, microglia in the prefrontal cortex and hippocampus displayed robust morphological changes indicative of an activated phenotype. Gene expression analyses of enriched hippocampal microglia showed elevated levels of several inflammation-associated genes, including HMGB1 mRNA levels after CUS, and increased HMGB1 expression in microglia overlapped with the development of depressive-like behaviors during stress exposure. To determine if stress-induced microglia alterations persisted after CUS, additional studies were performed in which rats were exposed to CUS then allowed to recover for 4 weeks. Here we show that microglia morphological alterations along with elevated HMGB1 expression are present 4 weeks after CUS cessation. Furthermore, changes in microglia were associated with recurrence of depressive-like behaviors during the recovery period. Specifically, exposure to short-term unpredictable stress during the recovery period caused recurrence of anhedonia in rats previously exposed to CUS. Importantly, short-term unpredictable stress did not cause significant behavioral changes in naïve rats. Taken together, these data suggest that initial CUS exposure can evoke alterations in microglia HMGB1 signaling that may contribute to increased vulnerability to depressive-like behaviors following subsequent stress exposure. Studies are being conducted to directly test this hypothesis. Supported by NIMH Grants MH045481 and MH093897, and the State of CT.
Disclosures: T.C. Franklin: None. E.S. Wohleb: None. R.S. Duman: None.

Poster

504. Mood Disorders Animal Models I

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 504.26/L3


Title: Anhedonia in the Chick Anxiety-Depression Model

Authors: *A. L. SALMETO-JOHNSON1, M. K. JOURDAN2, K. J. SUFKA2; 1Psychology, Graceland Univ., Lamoni, IA; 2Psychology, Univ. of Mississippi, Oxford, MS

Abstract: Anhedonia, the loss of pleasure in previously pleasurable activities, is one of the cardinal features of depression. To further validate the chick anxiety-depression model as a neuropsychiatric simulation, we sought to quantify this behavioral endophenotype as well as its pharmaceutical reversal. Assessment of anhedonia involved measures of social reinstatement in a straight alley maze, which had a mirror at the end to simulate the presence of another chick. A baseline measure was taken for all chicks. The next day chicks were tested again (No Test), or exposed to a test apparatus for 90 min either with 2 conspecifics and mirrors (Social) or individually (Isolated) to induce a depression-like state, prior to straight-alley maze testing. In general, both start and goal latencies were delayed in the Social and Isolated groups compared to the No Test group. Planned comparisons demonstrated a significant delay in start latency in the Isolated group compared to the No Test group (p = 0.029) and appeared delayed in Isolated compared to the Social group. Experiment 2 assessed the impact of Imipramine (0, 10, 15 mg/kg) administered prior to isolation apparatus exposure to alleviate the display of anhedonia as assessed in the straight alley maze. In general, start latency increased in all groups compared to the No Test group and was decreased in the Imipramine groups compared to the Vehicle group. Planned comparisons showed a significant decrease in start latency in the 15 mg/kg (p = 0.014) and approached significance with the 10 mg/kg Imipramine groups (p = 0.077) compared to the Vehicle group. Goal latency increased in all groups compared to No Test and was deceased in the Imipramine groups compared to Vehicle. Planned comparisons showed a decrease in the 15 mg/kg Imipramine group compared to the Vehicle group (p = 0.032), which is interpreted as a reduction in anhedonia. Results suggest the ability to assess anhedonia in chicks and that Imipramine is capable of alleviating the expression of anhedonia as assessed in this paradigm.

Title: Light modulates spatial learning and memory in an animal model of SAD

Authors: *J. E. SOLER, T. IKENO, L. YAN; Michigan State Univ., East Lansing, MI

Abstract: Light has profound effects on brain and behavior, which are best exemplified in Seasonal Affective Disorder (SAD). SAD is a major depressive disorder; SAD patients experience recurring symptoms i.e. depressed mood, anxiety and cognitive impairments during the fall and winter when there is less sunlight. The symptoms can be alleviated by bright light therapy before full remission takes place in spring and summer. The neural mechanisms mediating the effects of light on mood and cognition are not well understood. Our previous work has developed an animal model of SAD utilizing the diurnal Nile grass rats (*Arvicanthis Niloticus*) (Leach et al, 2013; Deats et al, 2014). We have found that grass rats show increased depression- and anxiety-like behaviors in a winter-like 12:12 hr Dim Light-Dark (DLD) condition compared to those in a summer-like Bright Light-Dark (BLD) cycle. The objective of the present study was to enhance the face validity of this model of SAD and to explore the neural substrates underlying photic modulation of mood, anxiety and cognition. Specifically we examined spatial learning abilities, as well as structural and functional changes in the hippocampus. Spatial learning and memory was assessed in animals housed in either BLD or DLD conditions using two hippocampal-dependent tasks: Novel Place Preference (NPP) and the Morris Water Maze (MWM). The results revealed that, although short-term spatial learning and memory were not affected by lighting conditions in both tasks, long-term spatial memory in the MWM was significantly impaired in grass rats housed in DLD. In the hippocampus, we found that the expression of brain-derived neurotrophic factor (*bdnf*) mRNA was significantly lower in DLD compared to BLD animals. In addition to the functional changes in the hippocampus, morphological changes are also being analyzed using Golgi staining. The behavioral data provide further evidence for the claim that light affects mood and cognition in the diurnal grass rats in a way similar to that seen in diurnal humans. The data on hippocampal function and morphology will provide a starting point for determining how light affects synaptic plasticity and neuronal morphology in this structure to modulate learning and memory in a diurnal species.
Disclosures: J.E. Soler: None. T. Ikeno: None. L. Yan: None.

Poster

505. Mood Disorders Animal Models II

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 505.01/L5


Support: SHRF EG, NSERC DG

Title: Serotonergic markers clustering in blood lymphocytes from animals exposed to corticosterone parallels the alterations found in depressed patients

Authors: *E. Y. FENTON\(^1\), R. ROMAY-TALLON\(^2\), M. A. MITCHELL\(^3\), T. RIVERA-BALTANAS\(^4\), K. LEBEDEVA\(^5\), L. E. KALYNCHUK\(^6\), J. OLIVARES\(^7\), H. J. CARUNCHO\(^2\);
\(^1\)Project Search and Evaluation, Ctr. for Drug Res. and Develop., Vancouver, BC, Canada; \(^2\)Col. of Pharm. and Nutr., \(^3\)Dept. of Psychology, \(^4\)Col. of Med., Univ. of Saskatchewan, Saskatoon, SK, Canada; \(^5\)Meixoeiro Univ. Hosp., Vigo, Spain

Abstract: Depression is a severe neuropsychiatric illness that affects roughly 16% of the population. Despite its high prevalence, the complex etiology and symptomatology of depression make understanding and treating it particularly difficult. As such, a great deal of effort has been placed on identifying new biological mechanisms of antidepressant efficacy, and biomarkers to help guide diagnosis and treatment. Two proteins of particular interest in this regard are the serotonin transporter (SERT) and the serotonin 2A receptor (5HT2AR), as they facilitate serotonin neurotransmission, they are primary targets of antidepressant medication and alterations in their expression and activity have been documented in depressed patients. We have recently shown that patients showing the greatest reduction in depressive symptoms after pharmacological treatment have larger SERT and 5HT2AR protein clusters per blood lymphocyte, compared to controls and other depressed patients. These patients also show a normalization of SERT and 5HT2A clustering, whereas other depressed patients do not. In light of this evidence, the aim of the current study was to identify whether similar alterations in SERT and 5HT2A clustering occur in a well-established rodent model of depression. To examine this, 40 male rats (20 for the SERT study and 20 for the 5HT2AR study) were administered the stress hormone corticosterone (CORT; 40mg/kg) for 21 days and depressive-like behaviour was assessed using the forced swim test. Blood was collected via cardiac puncture and
immunocytochemistry was used to examine SERT and 5HT2AR protein clusters in lymphocytes. As expected, CORT-treated animals showed a significant increase in depressive-like behaviours. Interestingly, the size of SERT and 5HT2AR clusters was increased significantly by 13% and 7% respectively, while the number remained unchanged. Moreover, a significant positive correlation was found between depressive-like behaviour and cluster size and a negative correlation was found between depressive-behaviour and cluster number. Together with our previous findings, these data suggest that SERT and 5HT2AR may be important biomarkers of depression, and that our rodent model of the disorder is a reliable way for us to examine this relationship more closely.


Poster

505. Mood Disorders Animal Models II

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 505.02/L6


Support: NIMH Grant MH093897

NIMH Grant MH14276

NIMH Grant MH106287

State of Connecticut

Title: Neural circuitry underlying the ketamine-like antidepressant effect of infralimbic prefrontal cortex optogenetic stimulation

Authors: *A. M. Thomas¹, E. S. Wohleb², R. J. Dileone², R.-J. Liu², G. K. Aghajanian², R. S. Duman²; ¹Dept. of Psychiatry, ²Yale Sch. of Med., New Haven, CT

Abstract: Ketamine is an antidepressant that promises to be more effective and faster-acting than any of the currently available depression pharmacotherapies, but its clinical use is limited by side effects. It is thus important to understand how ketamine acts in the brain in order to develop new therapies that utilize its mechanism of action. One key aspect of its mechanism that
is not well understood is the neural circuitry underlying its effects. Ketamine is known to increase glutamate release in the rodent medial prefrontal cortex (mPFC), which is critical to its antidepressant action. Part of the rodent mPFC, the infralimbic cortex (IL), is thought to correspond to the human anterior cingulate and ventromedial PFC, which have been shown through functional brain imaging to be differentially active in depressed patients compared to controls. Consistent with these data, our lab has demonstrated an antidepressant effect similar to that of ketamine by optogenetically inducing glutamatergic activity in the IL. Specifically, a single, one-hour 10-Hz laser stimulation of IL excitatory neurons infected with an rAAV2-CaMKIIα-ChR2(H134R)-EYFP construct produces an antidepressant effect lasting up to 2 weeks, as measured by the forced-swim test (FST) and novelty-suppressed feeding test (NSFT), which are measures of depression and anxiety. The effect is specific to the IL, as stimulation of the nearby prelimbic cortex does not produce an antidepressant effect. Using these optogenetic manipulations, we have sought to further characterize the circuitry underlying this antidepressant effect. Three brain areas in particular, the dorsal raphe, lateral habenula, and nucleus accumbens, have robust connections to the IL and are involved in the development of depressive features in rodents. Ongoing studies are assessing the importance of the IL projections to each of these brain areas, and how they relate to the observed antidepressant effect of IL stimulation, by analyzing the behavioral effects of optogenetic stimulation of light-sensitive axon terminals in each region after injection of the viral channelrhodopsin construct into the IL. Molecular studies are also being conducted to characterize changes in activity in these regions and to determine which cell types are the target of these projections. Supported by NIMH Grants MH093897 and MH14276, and the State of CT.


Poster

505. Mood Disorders Animal Models II

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 505.03/L7


Support: NIMH Grant MH045481

NIMH Grant MH093897

State of Connecticut
Title: Role of neuronal vascular endothelial growth factor signaling in the actions of antidepressants

Authors: *S. DEYAMA, X.-Y. LI, E. S. WOHLEB, S. DUTHEIL, A. BECKER, R. S. DUMAN;
Psychiatry, Yale Univ. Sch. of Med., New Haven, CT

Abstract: Growing evidence demonstrates that growth factors play a significant role in the pathophysiology and treatment of mood disorders. Our previous work has shown that vascular endothelial growth factor (VEGF) signaling contributes to the behavioral and neurogenic actions of typical antidepressants, notably the monoamine reuptake inhibitors. VEGF is a pleiotrophic growth factor expressed by neurons and glia, as well as vascular endothelial cells, and it is possible that the antidepressant actions of VEGF are mediated by one or more of these cell types. Here we examine the role of neuronal VEGF signaling by generating conditional deletion of either VEGF or Flk-1 (VEGF receptor 2) in neurons. CaMKII-Cre mice were bred with homozygous Flk-1 (VEGF receptor 2) or VEGF floxed mice. The resulting neuronal specific deletion of Flk-1 (Flk-1\(^{\text{NEURON}^{-/-}}\)) and VEGF (VEGF\(^{\text{NEURON}^{-/-}}\)) were tested in antidepressant responsive behavioral models, including the forced swim test (FST). The antidepressant effect of repeated desipramine (DMI) was blocked in Flk-1\(^{\text{NEURON}^{-/-}}\) or VEGF\(^{\text{NEURON}^{-/-}}\) mice. In addition, the antidepressant responses to chronic fluoxetine (FLX) or a single dose of ketamine were blocked in Flk-1\(^{\text{NEURON}^{-/-}}\) mice (VEGF\(^{\text{NEURON}^{-/-}}\) have not yet been tested). The locomotor response was not altered in either mutant line, indicating that the observed effects are not due to a general effect of neuronal Flk-1 or VEGF deletion on ambulation. These results indicate that neuronal VEGF signaling in the forebrain, the region where CaMKII drives expression of Cre, contributes to the actions of both typical and rapid acting antidepressants. To further define the forebrain region underlying these effects and to test the role of VEGF release, we have examined the medial prefrontal cortex (mPFC), a region implicated in the actions of ketamine, by intra-mPFC infusion of a VEGF neutralizing antibody (nAb). Infusion of the VEGF nAb into the mPFC significantly blocked the antidepressant effects of ketamine in the FST and in the novelty-suppressed feeding test, indicating that VEGF signaling in the mPFC plays an important role in antidepressant actions of ketamine. We are currently developing an approach to examine the effects of Flk-1 knockdown in CaMKII+ neurons in the mPFC via viral mediated expression of floxed Flk-1 shRNA in CaMKII-Cre mice. Together these studies will further characterize the cellular mechanisms underlying the antidepressant actions of VEGF signaling.

Disclosures: S. Deyama: None. X. Li: None. E.S. Wohleb: None. S. Dutheil: None. A. Becker: None. R.S. Duman: None.

Poster
Effect of repeated restraint stress on depression-like behavior and protein level of FKBP5 in the amygdala in rats

Authors: *T. IZUMI, R. GHEBREAB, C. WANG, Y. OHMURA, T. YOSHIDA, M. YOSHIOKA;
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Abstract: Major depression is a life threatening psychiatric disorder, and hyperactivation of HPA axis is one of the pathophysiological changes of it. The hippocampus and the medial prefrontal cortex inhibit the HPA axis, besides the amygdala activates it. Binding of FKBP5 decreases glucocorticoid receptor (GR) sensitivity to its ligand and alters intra-nuclear translocation. Here we investigated the effect of single and repeated restraint stress (RS) (3 hr/day) on depression- and anxiety-like behaviors using forced swimming test (FST) and elevated plus maze test (EPM) in rats. Moreover, we assessed the effect of repeated RS on the serum corticosterone by EIA, and on the GR and FKBP5 in the medial prefrontal cortex, hippocampus and amygdala by Western blotting. We performed above these assessments 2 weeks after repeated stress. Seven times RS increased depression-like behaviors (immobilization in FST) (P< 0.017). Locomotor activity and anxiety assessed by EPM were no change. Serum corticosterone was increased by seven times RS (P< 0.017). Protein level of GR was not changed, but that of FKBP5 was increased in the amygdala by seven times RS (P< 0.05). Hyperactivation of HPA axis and increase of FKBP5 in the amygdala may be related to RS-induced depression-like behaviors.

Program/#Poster#: 505.05/L9

**Topic:** C.16. Cognitive, Emotional, and Behavioral State Disorders

**Support:**
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- State of CT

**Title:** NR2B-containing NMDA receptors on prefrontal cortex interneurons and the rapid antidepressant effects of ketamine

**Authors:** *D. M. GERHARD*¹, E. S. WOHLEB², K. T. OTA², S. R. TAYLOR², M. R. PICCIOTTO², R. S. DUMAN²;
  ¹Psychology, ²Mol. Psychiatry, Yale Univ., New Haven, CT

**Abstract:** Major depressive disorder (MDD) is a growing public health concern with widespread effects on personal welfare, the economy and society. Recent efforts have focused on the therapeutic benefits of ketamine, an NMDA receptor antagonist that produces rapid and sustained antidepressant effects in treatment resistant patients. Rodent studies show that ketamine rapidly increases glutamate release, activates mTORC1 signaling and increases translation of synaptic proteins in the medial prefrontal cortex (mPFC) shortly following acute treatment. Additional studies suggest that ketamine exerts behavioral effects through blockade of NR2B-containing NMDARs. Selective NR2B antagonists activate mTORC1 signaling and produce antidepressant-like behavioral effects comparable to ketamine. Collectively, these studies point to blockade of NR2B-containing NMDARs as the critical mediator for the rapid antidepressant effects of ketamine. NR2B is expressed by excitatory glutamate expressing (CaMKII+) pyramidal neurons as well as inhibitory GABA-expressing (GAD67+) interneurons in the mPFC. To determine if ketamine exerts rapid antidepressant effects via blockade of NR2B on glutamatergic (CaMKII+) pyramidal neurons or GABAergic (GAD67+) interneurons in the mPFC we developed a viral construct that expresses a short-hairpin RNA targeting NR2B in a Cre-dependent manner. Preliminary results demonstrate that this shRNA construct reduces NR2B expression in cultured cells in a Cre-dependent manner. Moreover, we have found that viral-mediated NR2B knockdown in GAD67-Cre mice produces an antidepressant response and occludes the antidepressant effects of ketamine in the forced swim test. In contrast, preliminary studies indicate that the NR2B knockdown in CaMKII-Cre mice does influence immobility or block the actions of ketamine, although additional studies are needed. Immunohistological studies of subtypes of GABAergic interneuron show that parvalbumin (PV)- and somatostatin (SST)-expressing interneurons have varied distribution of NR2B expression in the mPFC, with lower levels of NR2B on PV neurons. Studies are currently being conducted to determine if NR2B knockdown in SST-Cre and PV-Cre lines blocks the on the antidepressant actions of ketamine.

Poster

505. Mood Disorders Animal Models II

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 505.06/L10


Support: NIMH Grant MH045481

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State of CT

Title: Ketamine reverses helpless behavior in rats exposed to acute mild stress

Authors: *D. LOPRESTO, T. C. FRANKLIN, M. J. GIRGENTI, R. S. DUMAN;
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Abstract: Ketamine, a noncompetitive N-methyl-D-aspartate (NMDA) glutamate receptor antagonist, produces rapid antidepressant effects in treatment resistant depressed patients. Molecular studies show that ketamine rapidly induces synaptogenesis and reverses stress-induced synaptic deficits by stimulating postsynaptic AMPA receptors and raising BDNF levels. This is thought to occur via blockade of GABAergic interneurons that results in a transient burst of glutamate. At the behavioral level, acute ketamine administration has been shown to produce robust antidepressant effects after chronic unpredictable stress exposure as well as in animals that have undergone intracranial surgery, another form of stress. Ketamine is also reported to produce antidepressant actions in naïve rodents in drug screening models, notably the forced swim test (FST). However, our recent FST studies of ketamine in naïve animals have been inconsistent, possibly due to environmental stressors resulting from different vendors, transport, or vivarium conditions. This discrepancy suggests that acute mild stress could provide a better, more consistent model to study the antidepressant effects of ketamine. To test this hypothesis, we determined if exposure to acute mild foot shock stress could unmask ketamine’s antidepressant properties. A 2 x 2 experimental design was used, where male Sprague-Dawley rats were exposed to mild foot shocks, which causes a two-fold increase in serum corticosterone levels, or remained in their home cage and received either vehicle or a single dose of ketamine (10 mg/kg, i.p.). The actions of ketamine were subsequently examined 24 hr later in a well-established
antidepressant model, the FST, a measure of behavioral despair that is reversed by acute antidepressant administration. Our results demonstrate that exposure to acute mild foot shock stress increases immobility time in the FST, and a single dose of ketamine significantly reverses immobility time and latency to immobility to levels in control, unstressed rats. Importantly, in agreement with our recent studies ketamine showed no antidepressant effects in naïve, unstressed rats in the FST, either in immobility time or in the latency to immobile posture. In conclusion, ketamine was able to reverse helpless behavior in the FST in rats exposed to mild foot shock stress but had no effect in naïve rats. Based on these findings, we conclude that prior exposure to a mild stressor provides a more consistent approach to uncover the antidepressant behavioral actions of ketamine.

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Poster

505. Mood Disorders Animal Models II

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Support: 1R01MH107183-01

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R01MH100616

Title: Chronic corticosterone-mediated dysregulation of microrna network in prefrontal cortex of rats: relevance to depression pathophysiology

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Abstract: Stress plays a major role in inducing depression, which may arise from interplay between complex cascades of molecular and cellular events that influence gene expression leading to altered connectivity and neural plasticity. In recent years, microRNAs (miRNAs) have
carved their own niche owing to their innate ability to induce disease phenotype by regulating expression of a large number of genes in a cohesive and coordinated manner. In this study, we examined whether miRNAs and associated gene networks play a role in chronic corticosterone (CORT; 50 mg/kg x 21 days)-mediated depression in rats. Rats given chronic CORT showed key behavioral features that resembled depression phenotype. Expression analysis revealed differential regulation of 26 miRNAs (19 upregulated, 7 downregulated) in prefrontal cortex of CORT-treated rats. Interaction between altered miRNAs and target genes showed dense interconnected molecular network, in which multiple genes were predicated to be targeted by the same miRNA. A majority of altered miRNAs showed binding sites for glucocorticoid receptor element, suggesting that there may be a common regulatory mechanism of miRNA regulation by CORT. Functional clustering of predicated target genes yielded disorders such as developmental, inflammatory, and psychological that could be relevant to depression. Prediction analysis of the two most prominently affected miRNAs miR-124 and miR-218 resulted into target genes that have been shown to be associated with depression and stress-related disorders. Altogether, our study suggests miRNA-mediated novel mechanism by which chronic CORT may induce depression phenotype in rats.

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**Poster**

**505. Mood Disorders Animal Models II**

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**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program/#Poster#: 505.08/L12

**Topic:** C.16. Cognitive, Emotional, and Behavioral State Disorders

**Support:** CNRS

University of Strasbourg

NARSAD

Institut UPSA de la Douleur

**Title:** Efferents of the mouse anterior cingulate cortex

**Authors:** *P. VEINANTE, C. FILLINGER, M. BARROT, I. YALCIN; INCI CNRS UPR3212, Strasbourg Cedex, France
Abstract: The anterior cingulate cortex (ACC) is known to be involved in chronic pain and depression in human and rodent. While the connectivity of ACC has been studied in primate and rat, a complete mapping of its efferents is still missing in the mouse which is a relevant model for preclinical studies. Thus, we analyzed the efferents of the mouse ACC by injecting anterograde tracers (biotin dextran amine, BDA, and Phaseolus vulgaris leucoagglutinin, PhaL) in the rostral and caudal parts of the dorsal (Cg1) and ventral (Cg2) quadrants of the ACC. As a whole, ACC was found to project strongly to cortical areas, thalamus, basal ganglia and associated structures, midbrain and pons. In addition to specific intra-ACC connections, main cortical targets included orbital, retrosplenial, associative parietal and secondary visual areas. A light projection was identified to the dorsal subiculum and postsubiculum. Subcortical forebrain projections were dense in the dorsal striatum and the claustrum, and moderate in the basolateral amygdaloid nucleus, lateral septum and medial septum/diagonal band. Main thalamic targets were the anteromedial, interanterodorsal, laterodorsal, mediodorsal, ventromedial, paracentral and reuniens nuclei; lighter projections occurred in centromedial, ventral anterior and rhomboid nuclei. The dorsal zona incerta was also strongly innervated by ACC. In the hypothalamus, a moderate projection was found in lateral and posterior areas. ACC projections to the midbrain were dense in the pretectal nuclei, superior colliculus, dorsolateral PAG, ventral tegmental area and substantia nigra. The anterograde labeling was further identified caudally in paramedian, median and dorsal raphe, oral pontine reticular nucleus, laterodorsal tegmental nucleus, ventromedial medulla and gigantocellular nucleus. Several differences in topography and/or labeling density were observed between Cg1 and Cg2 along the rostrocaudal axis, especially in cortical, striatal and thalamic targets, as well as in midbrain and pontine projections. While most of forebrain and thalamic efferents were reciprocal, excepted to the striatum, the extensive pontomesencephalic ACC projection appeared mostly unidirectional. These results disclose the organization of ACC outputs in mice and, along with our companion study of ACC inputs, emphasize its position at the center of a circuit relevant for pain and depression.

Disclosures: P. Veinante: None. C. Fillinger: None. M. Barrot: None. I. Yalcin: None.
Title: Rapid acting antidepressants cause activity dependent release of BDNF and stimulate mTORC1 signaling in primary neuronal cultures

Authors: *E. BANG, A. E. LEPACK, M. FUCHIKAMI, J. M. DWYER, A. TROG, M. BANASR, R. S. DUMAN;
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Abstract: Clinical studies have demonstrated that a single low dose of ketamine, a non-competitive NMDA receptor antagonist, can elicit rapid and long lasting antidepressant effects in treatment resistant depressed patients. Our lab has demonstrated that ketamine rapidly activates the mammalian target of rapamycin complex 1 (mTORC1) pathway in the medial prefrontal cortex (mPFC) of rodents, which is required for the synaptogenic and behavioral actions of ketamine. In addition, activation of mTORC1 and the antidepressant behavioral effects of ketamine are blocked by pretreatment with an AMPA receptor antagonist, indicating that neuronal activation is required. A role for BDNF is supported by studies demonstrating that the behavioral actions of ketamine are blocked by infusion of an anti-BDNF antibody into the mPFC and that the response to ketamine is blocked in Val66Met knock-in mice, which have impaired activity dependent release of BDNF (Liu et al., 2012). Taken together, these findings indicate that the behavioral responses to ketamine are mediated by activity dependent release of BDNF and activation of mTORC1 signaling. The aim of the present study was to directly test if ketamine increases BDNF release and activates mTORC1 signaling in primary neuronal cultures. The results demonstrate that ketamine produces a rapid (15 - 30 min) and dose dependent (10 to 500 nM) increase in mTORC1 signaling and BDNF release, and that these effects are dependent on activation of AMPA receptors. Moreover, preliminary studies demonstrate that other rapid acting antidepressants, including scopolamine (a muscarinic receptor antagonist), LY341495 (an mGluR2/3 receptor antagonist), and GLYX13 (a glycine site partial inverse agonist) also increase BDNF release and increase mTORC1 signaling in primary cultures, and that these effects are dependent on AMPA receptor activation. Finally, incubation with ketamine increases the complexity of dendrites in primary neuronal cultures, possibly as a result of increased BDNF release. Studies are being conducted to test the actions of the other rapid acting agents on dendrite morphology, and to test the role of AMPA receptor activation and BDNF signaling. Together these studies demonstrate that ketamine and rapid acting antidepressants cause activity dependent release of BDNF that leads to synaptogenic responses.

Transcriptional networks of resilience in a mouse model of depression

Major Depressive Disorder (MDD) is one of the most debilitating psychiatric disorders worldwide. Despite this, our understanding of the underlying mechanisms of MDD is far from complete. While genetics is believed to confer up to 40 percent of the risk for developing MDD, no genetic loci have emerged as genome-wide significant from a multitude of GWAS studies. Animal models of MDD, such as chronic social defeat stress (CSDS) in mice, have been successful in identifying molecular determinants of susceptibility and resilience to stress, but experiments to date have primarily focused on single genes and biochemical pathways as opposed to gene networks. Consequently, development of targeted antidepressants has been limited and a large portion of MDD patients fail to respond to currently available therapeutics.

As such, a more comprehensive network-based approach to studying MDD is needed. In this study, we performed differential expression and Weighted Gene Co-Expression Network Analysis on transcriptional profiles obtained from key depression-related brain regions (nucleus accumbens, prefrontal cortex, basolateral amygdala, and ventral hippocampus) following 10-day of CSDS. We identified seven gene networks that show differential connectivity between mice resilient to CSDS and mice susceptible to CSDS. Four of these gene networks are also enriched for genes differentially expressed between resilient and susceptible mice. We have characterized key drivers within these four networks and identified upstream regulators of the networks that are putative master-regulators of resilience to CSDS. Furthermore, by assessing enrichment of known pharmacologic transcriptional signatures within these resilient-specific modules, we have generated predictions for novel therapeutic applications of existing drugs that may produce antidepressant effects by regulating network-level gene expression.
Mood Disorders Animal Models II

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 505.11/L15


Support: NIH grant MH191180

Title: Inhibitory modulation of orbitofrontal cortical activation on medial prefrontal cortex-amygdala information flow: Implication of interacting systems of obsessive-compulsive disorder and major depressive disorder

Authors: *C.-H. CHANG, A. A. GRACE; Dept. of Neurosci., Univ. of Pittsburgh, Pittsburgh, PA

Abstract: One to two thirds of patients with obsessive-compulsive disorder (OCD) display depressive symptoms. Consequently, major depressive disorder (MDD) is considered to be the major psychiatric comorbidity in OCD. The pathophysiology of OCD includes hyperactivity of the orbitofrontal cortex (OFC)-related circuitry, and it has been suggested that MDD arises due to dysfunction of the medial prefrontal cortex (mPFC)-amygdala pathway. However, how the OFC interacts with mPFC-amygdala information flow is largely unknown. In this study, we used in vivo extracellular single-unit recordings combined with local drug infusion in anesthetized rats to examine how OFC activation modulates amygdala neurons that fire in response to mPFC activation. Neurons in the amygdala that were responsive to mPFC stimulation (~50% evoked spikes) were first recorded for 10 min for baseline (BL; total 300 trials at 0.5 Hz stimulation rate), followed by intra-OFC local infusion of N-Methyl-D-aspartate (NMDA; 0.75µg/0.5µl), NMDA antagonist (2R)-amino-5-phosphonopentanoate (APV; 5µg/0.5µl), or vehicle (VEH; 0.5µl). All neurons (n = 8 in each group) were then recorded for another 40 min. We found that OFC activation with NMDA significantly decreased the ability of the mPFC to drive amygdala neurons immediately following local drug infusion (p < 0.05; first 5-min time block), while APV or VEH did not change the evoked probability compared to BL at all time points. We next assessed how neurons in the amygdala responded to mPFC electrical stimulation (BL; ~50% evoked spikes in 50 trials) with OFC stimulation preceding mPFC stimulation (50 trials each; OFC-mPFC stimulation delay: 10, 20, 30, 40, 50, and 100 ms). OFC modulatory gating was
tested in naive and OFC tetanized animals (200 trials at 20Hz). We found that OFC activation had a significant inhibitory gating (changes in evoked probability > 15% relative to BL) on mPFC-amygdala evoked response in the majority of the amygdala neurons recorded (n = 20 out of 23) at all latency delays relative to BL (all ps < 0.05), which was totally abolished with OFC tetanus (n = 8). Our results suggest that OFC activation exerted an inhibitory modulation of the mPFC-amygdala pathway. Potentiation of the OFC-related pathways, if such a condition is present in OCD, could result in a loss of OFC control over the mPFC-amygdala pathway, and thus may contribute to depressive-like symptoms.

**Disclosures:** C. Chang: None. A.A. Grace: None.

**Poster**

**505. Mood Disorders Animal Models II**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program/#Poster#: 505.12/L16

**Topic:** C.16. Cognitive, Emotional, and Behavioral State Disorders

**Support:** Roche Postdoc Fellowship (JB);
Swiss National Science Foundation

**Title:** Norepinephrine regulates hippocampal gene expression after acute stress

**Authors:** *J. BOHACEK*¹, M. ROSZKOWSKI¹, F. MANUELLA², L. VON ZIEGLER¹, I. M. MANSUY¹;
¹ETH Zurich, Zurich, Switzerland; ²Univ. Zurich, Zurich, Switzerland

**Abstract:** Acutely stressful experiences can trigger or exacerbate neuropsychiatric disease in humans, including post-traumatic stress disorder and anxiety disorders. The ability of stress to elicit such profound behavior changes depends on its ability to regulate gene expression in various brain regions. Indeed, acute stress elicits a highly complex, tightly orchestrated neuronal response involving multiple brain regions and various stress-signals, including neurotransmitters and hormone systems. How these stress-signals contribute to the transcriptomic response, however, remains poorly understood. To address this issue, we first established a mouse model in which a brief cold swim challenge induces strong anxiety on the elevated plus maze. Then, we assessed the transcriptomic profile in response to stress in the hippocampus, a key region involved in cognitive function, mood as well as stress-axis regulation. We find strong, genome-wide changes in gene expression involving signaling networks critical for neuronal function.
Using pharmacologic tools, we determined that the majority of the top-regulated genes is dependent on noradrenergic signaling, while none of these genes depends on the classic stress-hormones corticosterone and corticotropin releasing hormone. This demonstrates that norepinephrine plays a key role in regulating gene expression following acute swim stress exposure and suggests that it may be involved in the stress-induced increase in anxiety.

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pain induced depression. Neuropathic pain was induced by cuffing the right sciatic nerve in male C57BL/6J mice. Anxiodepressive-related behaviors were evaluated through the novelty suppressed feeding, marble burying, splash and forced swimming tests. Mechanical thresholds were determined using von Frey filaments. We performed *in vivo* electrophysiological single unit recordings and local field potential recordings in the ACC. Recorded neurons were filled with neurobiotin to allow histological analysis. Cuff animals displayed an ipsilateral mechanical allodynia, and developed anxiodepressive-like behaviors with time. Interestingly, mice spontaneously recovered from the mechanical allodynia after 3 months, but still presented the anxiodepressive-like phenotype. Recordings from animals with depression-like behavior show increases in overall firing rate and in the number of bursting events in the ACC. This effect was observed independently from the presence or recovery from the neuropathic allodynia. These findings are supportive of long-term physiological alterations within the ACC with chronic pain-induced depression.

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**Poster**

505. Mood Disorders Animal Models II

**Location:** Hall A

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**Topic:** C.16. Cognitive, Emotional, and Behavioral State Disorders

**Support:** NIMH Grant MH045481

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State of CT

Naurex Inc.

**Title:** GLYX13 increases mTORC1 signaling and synaptogenesis in the prefrontal cortex

**Authors:** *C. H. DUMAN*¹, D. LOPRESTO¹, R.-J. LIU¹, R. TERWILLIGER¹, E. BANG¹, S. DUTHEIL¹, J. DWYER¹, A. BECKER¹, J. BURGDORF², J. R. MOSKAL², G. K. AGHAJANIAN¹, R. S. DUMAN¹; ¹Dept. of Psychiatry, Yale Univ., New Haven, CT; ²Naurex Inc, Evanston, IL
Abstract: Studies demonstrating rapid antidepressant actions of NMDA receptor antagonists, notably ketamine, in treatment resistant depressed patients represent the most significant advances in the field of depression over the past 60 years. This work has stimulated research and drug development to identify additional novel agents with less side effects and abuse potential. Studies by Moskal and colleagues demonstrate that GLYX13, a partial agonist of the glycine site of the NMDA receptor, produces rapid antidepressant actions in rodent models and also in depressed patients but without the psychotomimetic and dissociative side effects of ketamine. Electrophysiological studies also demonstrate that GLYX13, like ketamine increases synaptic plasticity in rodent models. In the current study, we examined the influence of GLYX13 on signaling pathways and synaptic responses in the prefrontal cortex (PFC), effects that have been identified to play a significant role in the antidepressant behavioral actions of ketamine. The results demonstrate that a single dose of GLYX13 increases signaling molecules in the mechanistic target of rapamycin complex 1 (mTORC1) pathway, including increased levels of the phosphorylated and activated form of p70S6K1, as well as increased levels of phospho-ERK in the PFC. In addition, a single dose of GLYX13 increased the number and function of spine synapses in layer 5 pyramidal neurons in the PFC. In these studies, GLYX13 administration increased the frequency of hypocretin-induced excitatory postsynaptic currents (EPSCs), and increased the number and head diameter of dendritic spines on layer 5 neurons. Elevation of the hypocretin response, which is mediated by thalamocortical terminal projections, would be expected to enhance attention and cognitive behaviors that are subserved by these connections. GLYX13 administration did not increase 5-HT-induced EPSCs. The results demonstrate that the actions of GLYX13 overlap with those of ketamine, as well as other rapid acting agents (i.e., scopolamine), although there are also differences that could potentially be related to the lack of psychotomimetic or dissociative side effects. Studies are being conducted to further characterize the type of spines that are influenced by GLYX13 and examine the thalamocortical inputs to these layer V postsynaptic elements.

Disclosures: C.H. Duman: None. D. Lopresto: None. R. Liu: None. R. Terwilliger: None. E. Bang: None. S. Dutheil: None. J. Dwyer: None. A. Becker: None. J. Burgdorf: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Naurex Inc.. F. Consulting Fees (e.g., advisory boards); Naurex Inc. J.R. Moskal: A. Employment/Salary (full or part-time); Naurex Inc.. G.K. Aghajanian: None. R.S. Duman: 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.; Naurex Inc. F. Consulting Fees (e.g., advisory boards); Naurex Inc.

Poster

505. Mood Disorders Animal Models II
Location: Hall A

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Program#/Poster#: 505.15/L19


Support: CNRS
   University of Strasbourg
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   NARSAD

Title: Afferents to the mouse anterior cingulate cortex

Authors: *C. FILLINGER, M. BARROT, I. YALCIN, P. VEINANTE;
   Cnrs - Upr3212 (inci), Strasbourg Cedex, France

Abstract: Depression is the most common mood disorder estimated to become the foremost contributor to the worldwide burden of disease by 2030. Because mechanisms underlying depression are not well understood, there is a need to investigate in greater detail the anatomical circuitry of the depression. The anterior cingulate cortex (ACC) is known, in human and rodent, to play a major role in depression. The connectivity of ACC has been studied in primate and rat, but a complete mapping is still missing in the mouse. Thus, we analyzed the afferents to the mouse ACC by injecting retrograde tracers (Fluorogold, FG, and the beta-subunit of the choleric toxin, CTb) in the rostral and caudal parts of the dorsal (Cg1) and ventral (Cg2) quadrants of the ACC. This allowed us to highlight five principal groups of structures projecting to the ACC: (1) cortical areas, with numerous retrogradely labeled neurons principally in orbital, medial prefrontal, retrosplenial, parietal associative, primary and secondary sensory areas and hippocampus; (2) basal forebrain with a dense labeling found in the basolateral amygdaloid nucleus, the claustrum and the diagonal band of Broca (HDB); (3), the hypothalamus with moderate labeling in the lateral area and a denser one in premamillary region; (4), thalamus with labeled neurons found principally in anterior medial, lateral mediodorsal, central lateral, central medial and reunions/rhomboid nuclei; and (5) the brainstem with a retrograde labeling exclusively located in monoaminergic centers. In most cases, the ipsilateral staining was more important than the contralateral one, excepted in the thalamus where no contralateral labeling was observed. Only few differences in afferents were found among the four ACC quadrants, especially in the orbital cortex and dopaminergic centers. Comparing FG and CTb results, some staining differences in density were found brainstem centers. Reciprocal anterograde tracing was performed with biotine dextran amine (BDA), to confirm retrograde labeling and further investigate the laminar organization of inputs from selected afferents: the HDB, the substantia
nigra pars compacta, the median raphe, and the locus coeruleus. Finally, the neurochemical nature of inputs coming from HDB and brainstem centers was investigated by a double-labeling procedure, showing that only a part of these afferents were cholinergic or monoaminergic. These results highlighted specific inputs to the ACC and replace it in a circuit which may be a new target for the study of the depression.

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**Poster**

**505. Mood Disorders Animal Models II**

**Location:** Hall A

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**Program#/Poster#: 505.16/L20**

**Topic:** C.16. Cognitive, Emotional, and Behavioral State Disorders

**Support:** NIH grant MH086539

**Title:** Comparison of intermittent and continuous swim stress-induced behavioral depression: a levels of analysis approach


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**Abstract:** Prior exposure to inescapable stressors produces a depression of normal active behaviors (i.e., behavioral depression) in response to subsequent stress treatment. Swim stress paradigms (particularly cold-water swim) have been widely validated as models of behavioral depression, with the key endpoint being increased immobility during a forced swim test (FST). This phenomenon of behavioral depression is mediated in large part by serotonergic (5HT) and noradrenergic (NE) systems. The pattern of stressor administration (i.e., intermittent or continuous) is known to differentially recruit 5HT and NE. Intermittent swim stress (ISS) and continuous swim stress (CSS) in cold-water enhance behavioral depression in response to a subsequent FST, indicated by increased immobility. ISS typically exposes rats to 100 5-second forced swims (total swim of 8-min at 15°C water), while CSS typically exposes rats to 15-min forced swimming (19°C water). Total water exposure differences, coupled with differential water temperature makes direct comparisons between ISS and CSS paradigms difficult to interpret. Furthermore, the neurobiology of CSS has been examined at length, while only indirect evidence exists to describe the role of SHT and NE systems in ISS-induced behavioral despair. Therefore,
the current experiment examined the effects of ISS and an equivalent (8-min) CSS on subsequent FST reactivity. Behavioral (immobility), endocrine (corticosterone), and molecular (c-Fos expression in 5HT and NE neurons) markers were assessed in order to provide a levels of analysis view. Treatment with either ISS or CSS resulted in enhanced immobility. Interestingly, prior exposure to CSS, but not ISS, resulted in an increase of FST-induced CORT release and LC activity. Both ISS and CSS pretreatment resulted in enhanced double-immunostained 5HT neurons in the caudal region of dorsal raphé, but not in other subregions. Overall, the data demonstrate that ISS and CSS result in differential physiological and equivalent behavioral responses to a subsequent FST.


Poster
505. Mood Disorders Animal Models II

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 505.17/L21


Support: Strategic Research Program for Brain Sciences by the Ministry of Education, Culture, Sports, Science and Technology of Japan

Title: Development of the rat late-onset depression model related to white matter lesion

Authors: *H. ONO¹, H. IMAI¹, S. MIYAWAKI¹, S. MIYATA², H. NAKATOMI¹, M. MIKUNI², M. FUKUDA², N. SAITO¹;
¹Dept. of Neurosurgery, Grad. Sch. of Medicine, The Univ. of Tokyo, Tokyo, Japan; ²Dept. of Psychiatry and Neuroscience, Gunma Univ. Grad. Sch. of Med., Maebashi, Japan

Abstract: [Background] Late onset depression (LOD) gets familiar with growing aged society and a significant contributor to the global burden of disease. The occurrence of white matter hyperintensities on T2-weighted magnetic resonance images is more frequent in patients with LOD, compared with early-onset depression. This fact indicates that white matter lesions (WML) may provoke some stress vulnerability leading to depression. In this study, we have developed a selective WML rat model with restraint stress (RS) to evaluate the correlation between the WML and depression. [Method] Sprague-Dawley rats (302-380g, n=108) were used in this study. Selective WML was induced under general anesthesia with bilateral endothelin-1 injection. Animals were randomly assigned to 4 groups: WML with RS (group 1); sham operation with RS
Two weeks after surgery, group 1 and 2 animals received 2 hours of RS a day, for 13 days. Some animals in group 1 and 4 received escitalopram along the protocol. Body weight (BW) was recorded daily and blood samples were collected at three time points along the protocol. Animals underwent a forced swimming test (FST) on the day following the 13th RS day. Animals were euthanized after the FST, and brain sections analyzed. [Result] Conventional histopathology of the operated rat brain revealed the selective damage of the internal capsule. RS significantly suppressed weight gain in groups 1 and 2 compared with non RS groups. Moreover the change in BW over time in group 1 was significantly different from group 2. The body weight reduction in group 1 reversed with the administration of escitalopram. The corticosterone levels were elevated at the seventh stress day and returned to basal levels at the thirteenth day in group 1 and 2. The immobility time on the FST for group 1 was longer than that of other groups. [Discussion] Accompanied with WML, repeated RS induced a reduction in weight gain and prolongation of immobility time in FST. These results provide preliminary evidence that WML could influence stress vulnerability leading to depression. Additionally, selective serotonin reuptake inhibitor reversed the weight gain reduction. In order to use this model as depression rat model, further behavioral tests need to be added, but it is considered that this model represents some aspects of the depression related to the WML, and may have a potential to contribute to the near future aging society.

and late-stage BD and have attributed the changes to neuroprogression. Neuroprogression in BD is characterized by a progressive increase in the frequency of affective episodes, decreased response to medications, development of systemic inflammatory and oxidative stress markers, and decline in cognitive function. A relevant preclinical model of BD neuroprogression could offer insight into the pathophysiologic mechanisms underlying the progressive change in symptoms. To this end, we have modeled recurrent affective episodes in young adult male rats using multiple day exposure to D-amphetamine followed by drug-free intervals. To model a single mania episode and recovery period, we treated rats with D-amphetamine for 7-days, followed by a 7-day drug-free period. To model neuroprogression, we exposed rats to two, three, four, or five of these mania episodes, separated by recovery periods. Rats that experienced increased numbers of mania episodes had increased spontaneous locomotor activity and abnormalities in habituation memory, compared to rats that experienced fewer mania episodes. These findings demonstrate that this model may have face validity to neuroprogression in BD.


**Poster**

505. Mood Disorders Animal Models II

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 505.19/L23

**Topic:** C.16. Cognitive, Emotional, and Behavioral State Disorders

**Support:** SHRF EG

**NSERC DG**

**Title:** Differential effects of corticosterone on the colocalization of reelin and neuronal nitric oxide synthase in the adult hippocampus in wild type and heterozygous reeler mice

**Authors:** *R. ROMAY-TALLON*¹, T. RIVERA-BALTANAS⁴, L. E. KALYNCHUK², H. J. CARUNCHO³;
¹Dept. of Pharm. and Nutr., ²Col. of Med., ³Col. of Pharm. and Nutr., Univ. of Saskatchewan, Saskatoon, SK, Canada; ⁴Dept. of Psychiatry, Meixoeiro Univ. Hosp., Vigo, Spain

**Abstract:** Repeated corticosterone (CORT) treatment induces a deficit in dentate gyrus subgranular zone reelin-positive cells, in maturation of newborn neurons, and results in a consistent depressive-like behavior. However, the molecular mechanisms underlying these
processes are not known in detail. The purpose of the present study was to characterize the effect of three weeks of 20mg/Kg CORT injections in the number of reelin and neuronal nitric oxide synthase (nNOS), as well as their colocalization, in hippocampal regions in wild type (WTM) and heterozygous reeler mice (HRM). ANOVA analysis shows a CORT x genotype interaction in the density of reelin+ cells co-localizing nNOS in the dentate subgranular zone and stratum-lacunosum moleculare, and in the density of nNOS+ cells in the hilus. There is a main effect of CORT in the density of both reelin+ and nNOS+ cells in the dentate subgranular zone and hilus, and in reelin+ cells in the molecular layer and CA3 stratum radiatum; and a main effect of genotype on the co-localization of both markers in the dentate subgranular zone, and in the density of reelin+ cell sin the stratum lacunosum moleculare. These alterations suggest a possible interconnection between reelin and nNOS expression that is altered by repeated CORT treatment.


Poster

505. Mood Disorders Animal Models II

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 505.20/L24


Support: KAKENHI 24500445

Grant-in-Aid for Scientific Research on Innovative Areas 25116521

Title: The involvement of NOX1/nicotinamide adenine dinucleotide phosphate, reduced form oxidase in anxiety- and depressive-like behaviors induced by stress

Authors: *M. IBI, J. LIU, C. YABE-NISHIMURA;
Kyoto Prefectural Univ. Med., Kyoto, Japan

Abstract: While the involvement of reactive oxygen species (ROS) in neural functions has been reported, the source of ROS in the nervous system has not been clearly identified. In addition to mitochondria, nicotinamide adenine dinucleotide phosphate, reduced form oxidase (NADPH oxidase) is a major source of ROS generated in the nervous system. It is a superoxide-generating flavoenzyme composed of the catalytic subunit, NOX. Several homologues of NOX have recently identified including NOX1, a nonphagocytic form of the enzyme. We previously
reported that NOX1-derived ROS affect pain signaling under pathological conditions (J Neuroscience 28:9486-9494, 2008; J Neuroscience 31:18094-18103, 2011). However, the functional significance of NOX1 in brain function have not been elucidated. This led us to examine the role of NOX1 in a broad range of behaviors using mice deficient in Nox1 (Nox1-KO). There was no difference in the social behavior between wild-type mice (WT) and Nox1-KO. When the spatial memory was evaluated in the Morris water maze test, there was no difference between the genotypes. Nox1-KO did not show the impairment of memory consolidation in contextual and cued fear conditionings. No difference in spared nerve injury (SNI)-induced tactile allodynia was demonstrated between the genotypes. On the other hand, the SNI-induced anxiety-like behavior in elevated plus-maze (EPM) test was suppressed in Nox1-KO. When the anxiety-like behavior was evaluated in intact mice, both genotypes showed similar anxiety levels in EPM and open-field tests. However, increased anxiety-like behaviors demonstrated in WT following acute or chronic two hour-restraint stress, were markedly ameliorated in Nox1-KO. In the Porsolt forced swim test and tail suspension test in intact mice, both genotypes showed the similar immobility time. When mice were subjected to chronic social defeat stress or chronic corticosterone administration, the social behavior was significantly decreased in WT, but not in Nox1-KO. Interestingly, decreased level of BDNF mRNA in prefrontal cortex of WT following corticosterone administration was significantly suppressed in NOX1-KO. Taken together, ROS derived from NOX1/NADPH oxidase may play a key role in anxiety- and depressive-like behaviors induced by stress.

Disclosures: M. Ibi: None. J. Liu: None. C. Yabe-Nishimura: None.

Poster

505. Mood Disorders Animal Models II

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 505.21/L25


Support: Epigenomics Flagship Project

Fondazione Cariplo Grant 2013-0790

Title: Lysine-specific demethylase1 modulates anxiety-related behavior regulating stress-evoked transcription of immediate early genes

Authors: *F. S. RUSCONI1, B. GRILLO1, L. PONZONI1, S. BASSANI1, E. TOFFOLO1, L. PAGANINI1, A. MALLEI2, D. BRAIDA1, M. PASSAFARO3, M. POPOLI2, M. SALA1, E.
BATTAGLIOLI¹;  
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Abstract: Stress-related brain plasticity contributes to environmental adaptation. However, chronic stress can elicit maladaptive neuronal responses linked to mood and anxiety disorders. Epigenetic modulation of stress-evoked transcription has been suggested to play a critical role in translating stressful stimuli into anxious phenotype. Here we demonstrate the involvement of transcriptional corepressor Lysine-specific demethylase 1 (LSD1/KDM1A) and its dominant-negative splicing isoform neuroLSD1, in the modulation of epigenetic mechanisms underlying the acquisition of normal anxiety-related phenotype. We show that in mouse hippocampus LSD1 and neuroLSD1 interact with the transcription factor SRF, setting the basal chromatin state of the SRF-targeted immediate early genes egr1 and c-fos. Indeed, we found that in mice complete lack (neuroLSD1KO mice) or reduction (neuroLSD1HET mice) of neuroLSD1 expression resulted in a low anxiety-like behavior. On a molecular point of view, neuroLSD1 mutant mice display reduced levels of positive histone marks at the egr1 and c-fos promoters dampening their stress-induced transcription. Interestingly, systemic administration of HDAC inhibitor SAHA to neuroLSD1KO mice restored egr1 and c-fos promoter chromatin structure as well as the normal behavioral phenotype. Therefore, proper IEGs transcriptional response to stress is instrumental to shaping the physiological anxiety profile. Remarkably, LSD1 expression itself is acutely increased by stress, involving LSD1 not only as a molecular transducer of stress stimuli, but also as a stress-response modifier. Our data provide a rationale to use LSD1 as a pharmacological target in the treatment of mood and anxiety disorders.


Poster
505. Mood Disorders Animal Models II

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 505.22/L26


Support: MH045481

MH093897
State of CT

**Title:** High fat food induces anxiety and anhedonia: first steps towards identifying the common neural pathological mechanisms linking type 2 diabetes and depression in rat models

**Authors:** *S. DUTHEIL, K. T. OTA, E. S. WOHLLEB, R. S. DUMAN; Yale Univ., New Haven, CT

**Abstract:** According to the World Health Organization, type 2 diabetes (T2D) and depression will have the greatest health care burdens worldwide by 2025. Increasing evidence suggests the existence of a bidirectional relationship, but the pathological cross-signaling underlying the increased risk of developing these two disorders remain unknown. In this regard, the current study seeks to identify important genes and intracellular cascades that work in concert under a chronic high fat diet (HFD) exposure, mimicking T2D in a rat male model. The results show that chronic HFD intake leads to glucose intolerance and alterations of the mammalian target of rapamycin complex 1 (mTORC1) pathway. The mTORC1 pathway integrates signaling from growth factors, energy, and nutrient levels to regulate translation and new protein synthesis. Such alterations under HFD exposure suggests that protein synthesis may be affected and could contribute to behavioral impairments. Interestingly, animals exposed to HFD develop anxiety-like, anhedonia and cognitive deficits that are similar the effects of chronic stress models of depression. We also found increased neuronal and peripheral corticosterone levels, enhanced pro-inflammatory cytokines levels (i.e. TNFα, IL-1β, IL-6), and alterations of Toll-Like receptor expression in the hippocampus of HFD rats. Hence, our findings provide a coherent picture showing how chronic HFD exposure causes multiple complications in the brain, thereby affecting intracellular signaling and gene expression that underlie behavior. We will also present data using pharmacological approaches to decrease the immune response via blockade of the purinergic receptors or to test the ability of the rapid acting antidepressant ketamine to reverse the behavioral and inflammatory effects elicited by HFD. This study is a first step towards understanding the mechanisms linking diet-induced diabetes to depression and should help to narrow the range of risk factors serving as a bridge between these two devastating disorders. In the future, early interventions targeting these disrupted pathways could help to decrease the prevalence of mood disorders in diabetic patients and reciprocally, then minimizing the burden associated with these two conditions.

**Disclosures:** S. Dutheil: None. K.T. Ota: None. E.S. Wohleb: None. R.S. Duman: None.

**Poster**

**505. Mood Disorders Animal Models II**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM
Title: Treatment with the rapid acting antidepressant ketamine accelerates fear extinction in rodents

Authors: *M. J. GIRGENTI*¹, D. LOPRESTO², J. R. TAYLOR², R. S. DUMAN²;
¹Physiol. and Neurobio., ²Psychiatry, Yale Univ. Sch. of Med., New Haven, CT

Abstract: Impaired fear extinction contributes to the persistence of post-traumatic stress disorder (PTSD) symptoms. To date, few pharmacotherapies have demonstrated sufficient efficacy in PTSD. As such, the development of novel agents that improve the extinction of fear memory is warranted. There is mounting evidence for the role of glutamate in mediating stress responsiveness, the formation of traumatic memories, and the pathophysiology of PTSD, raising the possibility of identifying novel therapeutic interventions for this disorder. In this regard, recent clinical studies demonstrate that infusion of the rapid acting antidepressant ketamine, a glutamate NMDA receptor antagonist, rapidly and significantly reduces symptom severity in PTSD patients. Here, we used a rodent model of fear conditioning to test the effects of ketamine on the rate of fear extinction as well relapse and to study the underlying neurobiology of this response. Rats received ketamine (10 mg/kg; i.p.) or saline twenty-four hours after fear conditioning (foot shock paired with an auditory cue) and the next day were subjected to an extinction-training paradigm (exposure to auditory cue without foot shock). We found that ketamine treatment did not influence conditioned freezing responses during the initial extinction training, but that freezing behavior was significantly lower the next day during the extinction recall session. We also observed that a combination of ketamine treatment and extinction training caused activation of cFos in the medial prefrontal cortex (mPFC) and a subsequent decrease in cFos in the amygdala after the second day of extinction recall. In addition, the enhancement of extinction learning with ketamine is associated with activation of mTORC1 signaling in mPFC. The enhancement in fear extinction recall parallels earlier work showing that ketamine induces synaptogenesis and plasticity in the mPFC- a brain region instrumental in the acquisition and retrieval of extinction. Taken together, our findings support the hypothesis that the formation of new neuronal circuits by ketamine enhances the recall of extinction and could represent a novel approach for the treatment of PTSD and other fear disorders.

Title: Social bonds, cardiac function, and serotonin: An investigation using the prairie vole

Authors: *N. MCNEAL, A. DAGNER, E. IHM, M. WOODBURY, W. COLBURN, J. WARDWELL, A. J. GRIPPO;
Psychology, Northern Illinois Univ., Dekalb, IL

Abstract: Social relationships positively influence psychological and biological function in humans and other mammals. The disruption of an individual's social environment can adversely impact mental and physical health. The prairie vole is a useful laboratory model to investigate the neurobiological mechanisms linking social stress to deleterious changes in health because these animals form monogamous pair bonds, and display cardiac and hormonal regulation in a manner similar to humans. Male-female prairie vole bond disruption induces depression-relevant behaviors and disrupted regulation of the cardiovascular and endocrine systems. We hypothesized that antidepressant drug treatment (sertraline; Zoloft) would buffer the deleterious changes in behavioral and cardiac function during social isolation from an opposite-sex partner. To investigate this, adult male prairie voles were implanted with wireless transmitters for electrocardiographic recordings. Following recovery, male prairie voles were paired with an unrelated female partner for 5 days, followed by isolation from the female for 20 days. After 5 days of isolation, males received either sertraline or vehicle treatment for the remaining 15 days of the experiment. Finally, measures of depressive behaviors were conducted 24 hours apart on the final 2 days of the experiment. Preliminary results indicate drug treatment was limited in its ability to buffer the negative cardiac and behavioral changes associated with social stress. All male prairie voles (regardless of drug treatment) displayed a significant (P<0.05) increase in heart rate during isolation. Drug treatment tended to decrease the depression-relevant behavior and cardiac function in the tail-suspension test; however, during the forced swim test this pattern was reversed. These results demonstrate that disruption of social bonds in prairie voles deleteriously influenced neurobiological regulation of behavior and cardiac function, but that
sertraline was not entirely effective in ameliorating social isolation-induced negative changes. Continued use of this rodent model will improve our understanding of the associations among negative social experiences, depressive behaviors, and physiological health.


Poster

505. Mood Disorders Animal Models II

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 505.25/L29


Support: NIMH

HDRF

BBRF

Title: Circuit-wide transcriptional profiling reveals opposing prefrontal cortical and ventral-hippocampal gene co-expression networks regulating depression susceptibility in mice

Authors: *R. C. BAGOT*¹, H. M. CATES¹, I. PURUSHOTHAMAN¹, Z. S. LORSCH¹, D. M. WALKER¹, C. J. PEÑA¹, I. S. MAZE¹², E. A. HELLER¹, M. A. DOYLE¹, O. ISSLER¹, X. LIU¹, J. L. STEIN⁴, K. N. SCOBIE¹, R. NEVE³, L. SHEN¹, B. ZHANG³, E. J. NESTLER¹; ¹Fishberg Dept. of Neurosci., ²Dept. of Pharmacol. and Systems Therapeut., ³Dept. of Genet. and Genomic Sci., Icahn Sch. of Med. At Mount Sinai, New York, NY; ⁴Dept. of Neurology, David Geffen Sch. of Med., UCLA, Los Angeles, CA; ⁵MIT, Cambridge, MA

Abstract: Recent functional studies suggest that opposing alterations in prefrontal cortex (PFC) and ventral hippocampus (VHIP) neuronal activity regulate susceptibility to chronic social defeat stress (CSDS) (Bagot et al., Nat Commun, in press), a highly validated mouse model of depression. However, the molecular mechanisms mediating depression-associated functional alterations in these brain circuits are largely unknown. We performed RNA-sequencing on multiple brain regions, including PFC and VHIP, from control animals and mice susceptible or resilient to CSDS at multiple time points after defeat. We employed an intersectional bioinformatics approach combining differential expression analysis with weighted gene co-expression network analysis and key-driver analysis to identify novel transcriptional networks regulating depression susceptibility. We used viral-mediated over-expression of identified
network hub-genes in mice exposed to CSDS and assessed effects on depression associated behavioral assays. We identified two susceptible-specific gene co-expression networks that exhibited significant enrichment of oppositely regulated differentially expressed genes in PFC versus VHIP. Both networks were significantly enriched for neuronal-specific genes and gene ontology analysis indicated relevant functions including synaptic transmission and cell-adhesion. Viral-mediated over-expression of hub genes in each network confirmed bioinformatic predictions, inducing increased defeat-elicited social avoidance in VHIP and reduced social avoidance in PFC. These results demonstrate that opposing regulation of gene co-expression networks in PFC and VHIP mediates susceptibility to social defeat stress. Moreover, validation of bioinformatically predicted hub-genes validates the utility of a systems biology approach in identifying novel transcriptional mechanisms that mediate depression-like behavior.


**Poster**

**505. Mood Disorders Animal Models II**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program# Poster#:** 505.26/L30

**Topic:** C.16. Cognitive, Emotional, and Behavioral State Disorders

**Support:** T32 MH14654

R01 MH086599

**Title:** Exposure to a novel environment inhibits nucleus accumbens dopamine response to palatable food in mice

**Authors:** *S. A. ROBINSON, T. E. HILL-SMITH, I. LUCKI;
Univ. of Pennsylvania, Philadelphia, PA

**Abstract:** Exposure to a novel environment inhibits nucleus accumbens dopamine response to palatable food in mice Shion A. Robinson¹, Tiffany E. Hill-Smith², and Irwin Lucki²³ Departments of Neuroscience¹, Pharmacology², and Psychiatry³ University of Pennsylvania, Philadelphia, PA, 19104. Anhedonia is a hallmark symptom of several psychiatric disorders, including depression, schizophrenia and post-traumatic stress disorder. Though the term
encompasses both consummatory and motivational components, clinical studies indicate that anhedonic patients display greater deficits in motivational behaviors as opposed to perception of pleasure. Given the high presentation of anhedonia in stress-related psychiatric disorders, we were interested in investigating the effects of stress on motivational drive for obtaining natural rewards. In these studies we implemented the use of C57BL/6J (C57) mice in assessing the neurochemical correlates underlying approach behavior for palatable food. Previous studies in our lab have found that in contrast to rats, C57 mice are neophobic and do not immediately approach palatable food. Instead, these mice require repeated exposures in a controlled environment to exhibit approach behavior and consume the food. Using in vivo microdialysis, we characterized the effect of palatable food exposure on dopamine release in the nucleus accumbens (NAc), a region known to play a prominent role in food reward behavior. We found that animals trained to consume food in the testing chamber exhibited a significant 30% elevation in dopamine release in the NAc when food was presented (p < 0.05). However, if a bright light and novel scent was presented during food exposure, dopamine release in response to the food reward was blocked. Furthermore, animals in this condition took longer to approach the food compared to animals in the non-aversive condition. Lastly, among the animals there was a significant correlation between dopamine release and latency to approach the food (r = -0.6358, p < 0.05), but not with amount of food consumed. Together, these data indicate that in C57 mice, the mild stress of exposure to novelty reduces motivational drive to approach palatable food via alterations in NAc dopamine responsiveness to the food reward. Thus, stress-induced dysregulation of dopaminergic activity in response to natural rewards may be an underlying cause of anhedonia. This research is supported by NIH/NIMH sponsored grants T32 MH14654 and R01 MH086599

Disclosures: S.A. Robinson: None. T.E. Hill-Smith: None. I. Lucki: None.

Poster

505. Mood Disorders Animal Models II

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 505.27/L31


Title: Altered dendritic spine plasticity in a mouse depression model

Authors: *L. H. L. NG¹, R. C. C. CHANG², C. S. W. LAI¹; ¹Department of Physiol., ²Department of Anat., The Univ. of Hong Kong, Hong Kong, Hong Kong
**Abstract:** Structural atrophy and functional deficit of prefrontal cortex (PFC) have been consistently reported in patients of major depressive disorder. In animal models of depression, chronic stress reduced both the complexity and density of dendritic spines in PFC, which can be reversed by antidepressants treatment. It has been found that ketamine, a NMDA receptor blocker, can exert rapid antidepressant effect at a single, sub-anaesthetic dose. Ketamine can also reverse chronic stress-induced spine loss through the induction of synaptogenesis in animal depression models. Nevertheless, data on the immediate and long-term effect of ketamine in dendritic spine plasticity is lacking. On the other hand, previous report showed ketamine increased cortical excitability through the disinhibition of pyramidal neurons by preferential suppression of cortical interneurons. However, the involvement of cortical interneurons in the antidepressant effect of ketamine remains elusive. In this study, we used in vivo two-photon transcranial imaging of 1 month-old male Thy1-YFP H line mice to investigate the dendritic spine plasticity in the chronic restraint stress (CRS) depression model. We found that CRS altered dendritic spine plasticity of layer V pyramidal neurons and reduced parvalbumin (PV) interneurons immunoreactivity in the frontal cortex. We will further investigate the short-term and long-term effect of ketamine on the dendritic spine plasticity, and the potential involvement of PV interneurons in the CRS depression model.

**Disclosures:** L.H.L. Ng: None. R.C.C. Chang: None. C.S.W. Lai: None.

**Poster**

**505. Mood Disorders Animal Models II**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 505.28/L32

**Topic:** C.16. Cognitive, Emotional, and Behavioral State Disorders

**Support:** NIH Grant MH045481

NIH Grant MH093897

State of Connecticut

**Title:** M1-type muscarinic acetylcholine receptors on prefrontal cortex interneurons mediate the rapid antidepressant effects of scopolamine

**Authors:** *E. S. WOHLBEB, K. T. OTA, D. M. GERHARD, J. M. DWYER, S. R. TAYLOR, M. R. PICCIOTTO, R. S. DUMAN; Yale Univ., New Haven, CT
Major depressive disorder (MDD) is a recurring psychiatric illness that causes significant health and socioeconomic burden. Clinical reports revealed that the muscarinic acetylcholine receptor antagonist, scopolamine, produces rapid antidepressant effects in individuals with MDD. Moreover, studies in our lab show that the antidepressant behavioral effects of scopolamine are due to increased glutamate release, activation of mTORC1 signaling, and enhanced dendritic spine density on pyramidal neurons in the medial prefrontal cortex (mPFC). Further studies in the lab suggest that scopolamine exerts behavioral effects through blockade of M1-type muscarinic acetylcholine receptors (M1-AChR). For instance, antagonists selective for M1-AChR activate mTORC1 signaling and promote antidepressant responses comparable to scopolamine. These findings indicate that the cellular trigger for the rapid antidepressant effects of scopolamine is blockade of M1-AChR in the mPFC. M1-AChR is expressed by excitatory, glutamate-expressing (CaMKII+) pyramidal neurons along with subsets of inhibitory, GABA-expressing (GAD67+) interneurons in the mPFC. To determine if scopolamine exerts rapid antidepressant effects via blockade of M1-AChR on CaMKII+ or GAD67+ neurons in the mPFC we developed a viral construct that expresses a short-hairpin RNA targeting M1-AChR in a Cre-dependent manner. Here we show that viral-mediated M1-AChR knockdown in GAD67-Cre, but not CaMKII-Cre, mice blocks the antidepressant effects of scopolamine in the forced swim and novelty suppressed feeding tests. Closer examination of GAD67+ interneuron subsets through immunohistology showed that parvalbumin (PV+) and somatostatin (SST+) interneurons have varied distribution and M1-AChR expression in the mPFC. Furthermore, viral-mediated M1-AChR knockdown in SST-Cre mice promoted a pro-depressive behavioral response and M1-AChR knockdown in SST, but not PV, interneurons of the mPFC attenuated the rapid antidepressant effects of scopolamine. These data indicate that M1-AChR on SST+ interneurons play a pivotal role in the rapid antidepressant actions of scopolamine. Supported by NIMH Grants MH045481 and MH093897, and the State of CT.

Title: "Nature and nature” in a genetic model of depression

Authors: *N. S. MEHTA, S. L. WERT, C. MORLEY, E. N. GRAF, E. E. REDEI;
Northwestern Univ., Chicago, IL

Abstract: “Nature and nurture” in a genetic model of depression Neha S. Mehta, Stephanie L. Wert, Claire Morley, Evan N. Graf, Eva E. Redei In this study, we sought to discover whether nurture in the form of environmental enrichment (EE) could overcome the negative effects of nature: the genetic causes of depressive behavior in a rat model of depression. This genetic rat model was developed in our lab from an accepted animal model of depression, the Wistar Kyoto (WKY) rat strain. Selective breeding of WKYs, based on immobility extremes in the forced swim test (FST), led to the Wistar Kyoto Less Immobile (WLI, control) and the Wistar Kyoto More Immobile (WMI, depression model) inbred strains, currently at their 35th generation. These strains contributed significantly to the discovery of blood transcriptomic markers of major depressive disorder (MDD). The thereby developed blood transcriptomic markers discriminated adolescent and adult subjects with MDD from their matched controls. In the current study, adult male WMIs and WLIs were either provided EE for 30 days or kept in their regular laboratory environment. Depression-like behavior decreased in both strains by EE, as measured by reduced immobility in the FST, with no change in anxiety-like behavior in the OFT. Nevertheless, WMIs still showed greater despair-like behavior post-EE, when compared to WLIs. Blood and hippocampal levels of transcripts that discriminated MDD subjects from controls in the clinical studies changed more in the WLIs than in WMIs. EE-induced changes in blood transcript levels of Ras association and pleckstrin homology domains 1 (Raph1), and hippocampal expression of Adenylate cyclase 3 (Adcy3) and Family with sequence similarity 214, member B (Kiaa1539) paralleled the changes in FST behavior post-EE in both strains. We propose that the maintained greater immobility of the WMIs post-EE is solely due to genetic factors. FST itself is a behavioral measure, but also an acute stressor. Indeed, FST altered the expression of several genes in the blood and in the hippocampus of WMIs and WLIs compared to those in behaviorally naïve animals. Thus, the attenuated immobility in both strains could be due to the EE-induced reduction of the FST-induced acute stress effect. The data suggest that an enriched environment can in fact positively impact depressive behavior, likely via reducing the environmental/stress contribution to its etiology, without influencing genetic susceptibility. Supported by the Davee Foundation.


Poster

506. Cocaine: Reward, Sensitization, and Locomotion

Location: Hall A
Title: Effect of neuron-specific deletion of Rbfox1 on learning and cocaine-related behaviors in mice

Authors: *J. DRGONOVA¹, G. R. UHL²;
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Abstract: Rbfox1 (A2BP1; Fox1) is a member of the Rbfox family of splicing regulators that displays high levels of expression in brain. Molecular genetic studies in humans associate variants in Rbfox1 with vulnerability to substance dependence as well as epilepsy, schizophrenia and intellectual disability/ autism. Genes whose primary transcripts are differentially spliced in brain and/or differentiated stem cells include many that have been implicated in addiction and in glutamatergic neurotransmission. Previous studies have shown that the brains of CNS-specific Rbfox1 knockout mice were hyperexcitable and that the homozygous knockout mice display epileptiform activities. Here, we expand the behavioral characterization of these mice, adding novel data for heterozygous Rbfox1 mice that might more accurately model common human level- of-expression variation. Rbfox1 knockout mice exhibit impaired learning/memory, altered sensitivity to cocaine reward as assessed by conditioned place preference test and baseline and cocaine-induced locomotor activities that are indistinguishable from those in wildtype littermates. These behavioral observations are consistent with contributions of variants in this gene to human phenotypes that include vulnerability to substance use disorders. Support: NIH IRP (NIDA).

Disclosures: J. Drgonova: None. G.R. Uhl: None.

Poster

506. Cocaine: Reward, Sensitization, and Locomotion

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 506.02/L35

Topic: C.17. Drugs of Abuse and Addiction

Support: RO1-ES023839
Title: Relationship between altered vesicular monoamine function and complex behavior

Authors: *R. A. CLIBURN, K. LOHR, L. RAJAN, J. SCHROEDER, D. WEINSHENKER, G. MILLER;
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Abstract: Vesicular monoamine transporter 2 (VMAT2) is a presynaptic transmembrane protein which sequesters dopamine, norepinephrine, and serotonin from the cytosol into the vesicular lumen to prepare neurotransmitters for release. Our laboratory utilizes transgenic mice across a continuum of vesicular function: VMAT2-LO, -WT, and -HI mice, which contain 5%, 100%, and 200% of WT levels of VMAT2, respectively. As VMAT2 gene dose increases, both basal concentration of dopamine and stimulated release of dopamine increases. We hypothesized that this increase in VMAT2 function mediates a range of monoamine-mediated complex behaviors in mice, including fear behavior and the appetitive response to psychostimulants. As previously reported, VMAT2-LO mice display an anxiety- and depressive-like phenotype, whereas VMAT2-HI mice show improved outcomes in these tests as evidenced by reduced immobility time in a forced swim test (21.7%) and fewer marbles buried in a marble burying assay (38.7%) compared to WT mice. Furthermore, VMAT2-LO mice exhibit a 34% increase in freezing response compared to VMAT2-WT or -HI mice in a test of contextual fear conditioning. Furthermore, VMAT2-HI mice display a reduced (50%) preference for a cocaine-paired context in a test of conditioned place preference at 10 mg/kg, but show no difference in cocaine-induced locomotion when compared to WT mice. Alternately, VMAT2-LO do not display an altered preference for cocaine at 10 mg/kg, but do display a marked increase in cocaine-induced locomotion. The ability of altered VMAT2 to influence response to psychostimulants, anxiety, depression, and fear response suggests that therapeutic approaches aimed at modifying VMAT2 function may be of benefit in these conditions.


Poster

506. Cocaine: Reward, Sensitization, and Locomotion

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 506.03/L36

Topic: C.17. Drugs of Abuse and Addiction

Support: 2SC1GM084854-05A1
Title: VTA Lamotrigine microinfusions accelerate the development of cocaine sensitization

Authors: *B. SANTOS VERA*¹, F. ARENCIBIA-ALBITE¹, A. VAQUER-ALICEA¹, R. VÁZQUEZ-TORRES¹, C. E. MARÍA-RÍOS², A. MONTIEL-RAMOS², M. DEVARIE-HORNEDO³, C. A. JIMÉNEZ-RIVERA¹;


Abstract: Dopaminergic projections arising from ventral tegmental area (VTA) play a key role on drug induced effects in the mesocorticolimbic system. Chronic cocaine administration produces an increased VTA dopamine (DA) cell excitability. VTA DA neurons express a large hyperpolarization activated cation current, known as Ih, that contributes to the electrophysiological properties of neurons in the CNS. We hypothesized that Ih-enhances VTA DA cell activity during the development of cocaine sensitization. Cocaine sensitization is the progressive escalation of psychomotor responses that results with repeated cocaine administration. We performed intra VTA microinfusions of Lamotrigine (LTG), an Ih current enhancer. Male Sprague-Dawley rats (250-300g) were anesthetized and stereotaxically implanted with bilateral 26-gauge cannulas in the VTA. Animals were divided as following: Vehicle/Saline, LTG/Saline, Vehicle/Cocaine, LTG/Cocaine (microinfusions/ i.p. injections, respectively). Daily LTG microinfusions (0.1ug bilateral) were administered for 7 days. Cocaine treated rats (15 mg/kg, i.p.) were injected 10-15 minutes after the LTG microinfusion. Locomotor activity was recorded for 1 hour every day. On day 8, rats received a first cocaine challenge in the absence of LTG. After a 7 days withdrawal period, a second cocaine challenge was performed. Total ambulatory and stereotype activities were analyzed using One-Way ANOVA followed by Newman-Keuls Test. LTG/Cocaine rats showed a significant increase in the acute response to cocaine (p< 0.01). Moreover, these animals developed sensitization two days before the control group. Their locomotor response to cocaine during days 1-3 was significantly higher (p< 0.01) compared to Vehicle/Cocaine rats. However, during cocaine challenge, when no LTG was administered the LTG/Cocaine group’s locomotor activity was not significantly different from the Vehicle/Cocaine group. We postulate that LTG in the VTA, acting through the Ih current, enhances neural excitability caused by chronic cocaine administration. Taken together, our data suggest that an enhanced Ih current activity in the VTA may contribute to a greater susceptibility to cocaine neurobiological effects.
Title: Effects of PKMζ inhibitor (ZIP) on the initiation and expression of cocaine sensitization

Authors: *A. VAQUER-ALICEA1, R. VÁZQUEZ-TORRES2, B. SANTOS-VERA2, C. MARÍA-RÍOS4, A. MONTIEL-RAMOS4, M. DEVARIE3, M. VÉLEZ-HERNÁNDEZ5, T. C. SACKTOR6; C. A. JIMÉNEZ-RIVERA2;

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Abstract: Cocaine addiction induces long-lasting alterations in the mesocorticolimbic system; some of which may be mediated by the mechanisms of long-term potentiation (LTP). Persistent phosphorylation by protein kinase M zeta (PKMζ) mediates the maintenance of late-LTP (L-LTP). The myristoylated z-pseudosubstrate inhibitory peptide (ZIP) selectively inhibits atypical PKCs, including PKMζ, and reverses established L-LTP. Previous laboratory data showed that ZIP microinjection in the ventral tegmental area (VTA) (important for initiation) on day 5 of a cocaine behavioral sensitization protocol blocked initiation of sensitization but not expression. We hypothesized that uninterrupted neural signaling from the VTA to the nucleus accumbens (NAc) (important for expression) for 5 days before inhibiting PKMζ, triggered plastic changes in the NAc. In accordance with this hypothesis, ZIP microinjections into the NAc after 7 days of withdrawal blocked expression. These data indicate that VTA LTP plays a role in the initiation of sensitization and NAc LTP allows sensitization expression. To elucidate if VTA LTP is
essential for plasticity changes in the NAc to occur, we continuously interrupted VTA LTP formation and looked for changes in expression. For this, male Sprague-Dawley rats (250g) were stereotaxically implanted with bilateral cannulas into the VTA and were given daily ZIP microinjections for 5 days, 6 hours after cocaine i.p. injection followed by a 7 day withdrawal period and a cocaine challenge. Results showed a decrease in total and stereotypic locomotion on day 5, but not on challenge day. This suggests that PKMζ is important but not essential for the initiation phase and that NAc neuroadaptations can develop independently of VTA LTP. In order to see if these neuroadaptations in the NAcc can be naturally restored once interrupted, we inhibited PKMζ a week after a withdrawal period and allowed another 7 days of withdrawal. A cocaine challenge was given on day 20. We observed a persistent decrease in total and stereotypic locomotion by day 20, indicating NAc LTP disruptions during the maintenance phase of the cocaine sensitization protocol are long-lasting. Further studies regarding PKMζ’s role in cocaine-induced LTP formation in the reward circuit, will shed some light into the pathological mechanisms that underlie cocaine addiction.


Poster

506. Cocaine: Reward, Sensitization, and Locomotion

Location: Hall A

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Topic: C.17. Drugs of Abuse and Addiction

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Neuroscience Research Center, Medical College of Wisconsin

Title: A within-animal assessment of neural ensembles associated with novelty and cocaine

Authors: *N. N. NAWARAWONG1,2, M. J. MUELBL1,2, H. ZHU3, Y. LIM1,2, B. L. ROTH3, C. M. OLSEN1,2;
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Abstract: Novelty seeking is a personality trait associated with an increased vulnerability for substance abuse. In rodents, elevated novelty seeking has been shown to be a predictor for elevated drug self-administration and compulsive use. While previous studies have shown that both novelty and drugs of abuse have actions within similar mesocorticolimbic regions, little is known as to whether the same neural ensembles are engaged by these two stimuli. In this project, we wanted to determine the activation patterns associated with novelty and cocaine. Using the TetTag mouse model (a dual transgenic reporter line that allows for long lasting temporally controlled tagging of active neurons), we compared neurons engaged by cocaine and novelty seeking. We investigated the infralimbic and prelimbic prefrontal cortex, the nucleus accumbens (NAc) core and shell, the ventral hippocampus, and the basolateral amygdala for overlap between neurons associated with cocaine and novelty seeking, and found significant overlap, especially in the NAc core. To test the functional significance of overlap between neural encoding of novelty and cocaine, we are using the TetDREADD mouse model; a variant of the TetTag mouse that yields activity-dependent expression of Gi/o coupled DREADD receptors (hM4Di) in a temporally controlled manner. TetDREADD mice were trained to self-administer cocaine and novelty (operant sensation seeking: OSS) in different contexts to test the ability of silencing neurons engaged during one of these behaviors to affect expression of the other behavior. The data suggest that increasing Gi/o signaling in neurons engaged during cocaine self-administration can reduce OSS. Ongoing studies are further parsing the functional overlap of neurons involved in cocaine and novelty seeking.


Poster

506. Cocaine: Reward, Sensitization, and Locomotion

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

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Topic: C.17. Drugs of Abuse and Addiction

Support: IRP/NIDA/NIH

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Title: Behavioral and physiological effects of a novel kappa opioid receptor based DREADD in rats
**Abstract:** In the past decade, novel methods using engineered receptors have enabled researchers to manipulate neuronal activity with increased spatial and temporal specificity. One widely used chemogenetic method in mice and rats is the DREADD (designer receptors exclusively activated by designer drugs) system in which a mutated muscarinic G-protein coupled receptor is activated by an otherwise inert synthetic ligand, clozapine-n-oxide (CNO). Recently, the Roth lab developed a novel inhibitory DREADD in which a mutated kappa opioid receptor (KORD) is activated by the pharmacologically inert drug salvinorin B (SalB; Vardy et al., 2015). They demonstrated the feasibility of using KORD to study brain circuits involved in motivated behavior in mice. Here we used behavioral, electrophysiological, and neuroanatomical methods to demonstrate the feasibility of using the novel KORD to study brain circuits involved in motivated behavior in rats. In Exp. 1, we show that SalB dose-dependently decreased spontaneous and cocaine-induced locomotor activity in rats expressing KORD in midbrain (ventral tegmental area/substantia nigra). In Exp. 2, we show that SalB completely inhibited tonic firing in KORD-expressing putative dopamine neurons in midbrain. In Exp. 3, we used a ‘retro-DREADD’ dual-virus approach to restrict expression of KORD in ventral subiculum neurons that project to nucleus accumbens shell. We show that KORD activation selectively decreased novel context-induced Fos expression in this projection. Our results indicate that the novel KORD is an excellent tool to selectively inactivate brain areas and neural circuits in rat studies of motivated behavior.

Abstract: Generally, mental stress enhances irresistible impulse to addictive craving. However, the molecular mechanism is not fully understood. We reproduced this behavior in a mouse model, in which pre-exposure of mice to social defeat stress increased cocaine preference. Motivation for preference is well known to be controlled by dopamine neurotransmission in reward system network from the ventral tegmental area to nucleus accumbens. We therefore performed microdialysis to detect dopamine levels in the nucleus accumbens. Dopamine levels in the nucleus accumbens increased when mice were placed in cocaine-conditioned box, and the increase in dopamine levels was significantly enhanced after social defeat stress. As dopamine is known to be an inducer of oxidative stress, we measured the expression level of oxidative stress response proteins, and found that nuclear respiratory factor was significantly increased only when mice were treated with cocaine after social defeat stress. Furthermore, overexpression of dominant negative form of nuclear respiratory factor in nucleus accumbens suppressed cocaine preference after social defeat, as in the case of delta2deltaFosB overexpression (Ohnishi YN et al., 2015). Now we are trying to determine whether the increase in nuclear respiratory factor can be reduced by antioxidant-rich-foods.


Poster

506. Cocaine: Reward, Sensitization, and Locomotion

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 506.08/L41
**Title:** Systematic investigation of toxicokinetics and neurobehavioral effects of cocaine in zebrafish larvae

**Authors:** *K. T. KIRLA*1,2, K. GROH2,3, A. STEUER1, M. POETZSCH1, R. EGGEN2,4, K. SCHIRMER2,4,5, T. KRAEMER1;

1Inst. of Forensic Med., Univ. of Zurich, Zurich, Switzerland; 2Eawag, Swiss Federal Inst. of Aquatic Sci. and Technology, Dept. of Envrn. Toxicology, Duebendorf, Switzerland; 3ETHZ, Dept. of Chem. and Applied Biosci., Zurich, Switzerland; 4ETHZ, Inst. of Biogeochemistry and Pollutant Dynamics, Zurich, Switzerland; 5EPFL, Sch. of Architecture, Civil and Envrn. Engin., Lausanne, Switzerland

**Abstract:** The main aim of our research is to understand the response of zebrafish larvae to psychoactive substances in order to evaluate the suitability of this system for prediction of the effects of such compounds in mammals. Here, we focus on the neurobehavioral effects and toxicokinetics of cocaine, a central nervous stimulant. The neurobehavioral effects of cocaine were assessed at 5 days post fertilization (5 dpf) by adding the drug to the fish water, followed by monitoring the locomotor activity. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) was used to study the uptake, biotransformation and elimination over time. Internal drug distribution was studied by matrix-assisted laser desorption ionization mass spectrometry imaging (MALDI-MSI) and also by LC-MS/MS quantification of cocaine in dissected body parts. Cocaine caused a significant reduction in the locomotory activity at the concentrations above 5 µM, while no significant effects were observed at lower concentrations. In mammals, cocaine is known to induce hyperactivity at low doses and hypoactivity at high doses, but in zebrafish larvae, only hypoactivity was observed. The reasons for the lack of hyperactive responses in zebrafish larvae are not known, but we hypothesized that it might be due to the differences in exposure routes used for fish and mammals. Hence we performed toxicokinetics and the distribution assessment. LC-MS/MS analysis confirmed the uptake and biotransformation of cocaine in zebrafish larvae and showed gradual elimination after transfer into clean water and effective concentrations were comparable to those causing similar effects in mammals. MALDI-MSI was used for the first time to study the distribution of cocaine in zebrafish larvae, revealing accumulation in the trunk (melanophores) and in the head region; more precisely, in the brain, but also, surprisingly, in the eyes. In dissected brain, eyes and trunk, cocaine was detected in all the measured tissues, with a highest concentration found in the eyes. In conclusion, this study provides new insights into toxicokinetics concurrently with the neurobehavioral effects of cocaine and is the first of its kind to demonstrate the possibility of drug distribution analysis by MALDI imaging in zebrafish larvae.

**Disclosures:**

- **K. T. Kirla:** None.
- **K. Groh:** None.
- **A. Steuer:** None.
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- **R. Eggen:** None.
- **K. Schirmer:** None.
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Poster

506. Cocaine: Reward, Sensitization, and Locomotion

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 506.09/L42

Topic: C.17. Drugs of Abuse and Addiction

Support: ETF Grant ETF9262

Title: The role of DNA methylation and demethylation in the expression of cocaine-induced behavioral sensitization

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Abstract: Repeated administration of psychostimulants (such as cocaine) induces an enhanced behavioral response to subsequent drug exposure, a phenomenon known as behavioral sensitization, which can be separated into two components - induction and expression. Induction of sensitization refers to the progressive increase in locomotor activity during the repeated drug treatments. Expression of sensitization is demonstrated following challenge with a low dose of psychostimulant after a drug-free period. Behavioral sensitization is remarkably persistent phenomenon. In rodents, it can persist from months to years after drug treatment is discontinued. Persistent behavioral sensitization indicates that drug-induced short- and long-term changes in gene expression may be involved. Accumulating data suggest that epigenetic mechanisms such as DNA methylation (catalyzed by DNA methyltransferases - DNMTs) contribute to drug-induced transcriptional and behavioral changes. Recently, it has been reported that ten-eleven translocation (TET) enzymes TET1-3, which add a hydroxyl group onto the methyl group of 5-methylcytosine (5-mC) to form 5-hydroxymethylcytosine (5-hmC) participate in DNA demethylation process and might play a role in cocaine action (Feng et al., 2015). Our aims of this study were: 1) to assess the effect of cocaine treatment on DNMT and TET family transcripts levels in the nucleus accumbens (NAc), cerebellum and peripheral blood of mice in the induction and expression phase of sensitization; 2) to determine whether or not treatment with DNMT inhibitor RG108 may affect the expression of cocaine-induced behavioral sensitization. Using qPCR, we found that cocaine treatment in the induction and expression of sensitization causes DNMT1 and DNMT3A overexpression and TET family transcripts (mainly TET1, TET3) downregulation in the NAc and cerebellum of mice and these changes are tightly correlated with changes in peripheral blood. ELISA-based DNMT and TET activity analysis showed that cocaine treatment in the expression phase of sensitization increased DNMT and...
decreased TET hydroxylase activity levels in the NAc. Using LC-ESI-MS/MS assay, we measured global 5-mC and 5-hmC levels in the NAc and we found that cocaine treatment alters 5-mC and 5-hmC levels in the induction and expression phase of sensitization. Finally, we show that repeated bilateral into NAc treatment with RG108 affects the expression of cocaine-induced sensitization in mice. These data indicate that cocaine treatment alters the balance between methylation and demethylation processes in the NAc and may affect the development of behavioral sensitization.


Poster

506. Cocaine: Reward, Sensitization, and Locomotion

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 506.10/L43

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant R01 DA09580

Title: Glycogen synthase kinase 3 in the rat ventral hippocampus is necessary for the development of cocaine-induced behavioral sensitization

Authors: *J. L. BARR, E. M. UNTERWALD;
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Abstract: The ventral hippocampus is involved in drug-seeking behavior and psychostimulant-induced behavioral sensitization. We have previously demonstrated that rats exposed to repeated cocaine exhibit greater increases in both locomotor activity and dopamine efflux in the medial shell of the nucleus accumbens in response to NMDA stimulation of the ventral hippocampus. Furthermore, cocaine-sensitized rats exhibit a greater density of NR2B-containing NMDA receptors in the ventral hippocampus. Glycogen synthase kinase 3 (GSK3) is a significant mediator of many intracellular signaling pathways, including increases in NMDA receptor function and trafficking of NR2B-containing NMDA receptors to the cell surface. Therefore, the current study examined whether inhibition of GSK3 in the ventral hippocampus diminishes cocaine-induced locomotor activity and the development of locomotor sensitization. Male rats were bilaterally infused with vehicle or the selective GSK3 inhibitor SB216763 (1ng) into the ventral hippocampus, 20 min prior to cocaine (15 mg/kg, ip.) or saline (1ml/kg, ip.) once daily for five days. After a ten day abstinent period all rats were challenged with a cocaine injection in
the absence of microinfusions. Pretreatment with the selective GSK3 inhibitor SB216763 into the ventral hippocampus significantly reduced cocaine-induced activity on days 2-5. Further, pretreatment with SB216763 attenuated the development of sensitization to the locomotor-stimulating effects of cocaine. The role of GSK3 in the regulation of NMDA receptors in the ventral hippocampus of cocaine-sensitized rats is currently under investigation. Overall, the findings suggest that GSK3 activity within the ventral hippocampus is necessary for the development of sensitization following repeated cocaine exposure. NMDA receptors may represent an important target for cocaine-activated GSK3 in the ventral hippocampus.

Disclosures: J.L. Barr: None. E.M. Unterwald: None.

Poster

506. Cocaine: Reward, Sensitization, and Locomotion

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 506.11/L44

Topic: C.17. Drugs of Abuse and Addiction

Support: National Institute on Drug Abuse Drug Supply Program

Title: Behavioral sensitization following concurrent exposure to MDPV and cocaine in CF-1 mice

Authors: *M. D. BERQUIST, JR, L. E. BAKER;
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Abstract: In the United States, reports indicate the specific “bath salt” constituent, 3,4-methylenedioxypyrovalerone (MDPV), is one of the most frequently encountered synthetic cathinones by law enforcement agencies. Recent reports indicate MDPV’s neurochemical actions on the dopamine transporter (DAT) are comparable to those of cocaine, although MDPV is considerably more potent. Moreover, preclinical behavioral assays of abuse liability indicate MDPV has a high risk for abuse. Behavioral sensitization to the hyperlocomotor effects of psychomotor stimulants is suggested to reflect changes in brain pathways associated with drug addiction and abuse. Although other synthetic cathinones (e.g., mephedrone) have been reported to induce behavioral sensitization following repeated dosing in rodents, MDPV has not yet been examined in this paradigm to our knowledge. Additionally, there is a scarcity of preclinical substance abuse research using female experimental subjects. The current study assessed the extent to which repeated exposure to mixtures of MDPV and cocaine (COC) produce augmented motor-stimulant responses in mice. Adult male (N=48) and female (N=48) CF-1 mice were
randomly assigned to receive intraperitoneal injections of saline, COC (5.0 mg/kg), MDPV (0.5, 1.0, or 5.0 mg/kg), or MDPV (0.5, 1.0, 5.0 mg/kg) + COC (5.0 mg/kg) daily for 7 days. After a 10 day washout period, all animals were dosed with 5.0 mg/kg COC. Activity was assessed for 30 min prior to injections and continuously monitored for 60 min after injections on days 1, 7, and 17. The development of behavioral sensitization was evident following repeated dosing in male mice treated with 1.0 mg/kg MDPV, 0.5 mg/kg MDPV + 5.0 mg/kg, or 5.0 mg/kg MDPV + 5.0 mg/kg COC. None of the above treatments induced sensitization in female mice. After the washout period, both male and female mice treated with 1.0 or 5.0 mg/kg MDPV, and those treated with MDPV+COC mixtures displayed cross-sensitization to the locomotor stimulant effects of 5.0 mg/kg COC. These findings suggest concurrent use of synthetic cathinones and cocaine may increase their risk of abuse. Additional studies evaluating sex differences in behavioral responses to the synthetic cathinones may be warranted.

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Poster

506. Cocaine: Reward, Sensitization, and Locomotion

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 506.12/M1

Topic: C.17. Drugs of Abuse and Addiction

Support: NIDA

Title: A role for nucleus accumbens somatostatin interneurons in cocaine induced plasticity

Authors: *E. A. RIBEIRO1, B. JUAREZ1, R. BAGOT1, I. PURUSHOTHAMAN1, B. LABONTE1, E. CALIPARI1, J. FENG1, J. SCARPA1, H. CATES1, M. HESHMATI1, A. KASARSKIS1, S. RUSSO1, L. SHEN1, M.-H. HAN1, J. KOO2, E. NESTLER1; 1Friedman Brain Inst., Icahn Sch. of Med. At Mount Sinai, New York, NY; 2Korea Brain Res. Inst., Denu, Korea, Republic of

Abstract: The nucleus accumbens (NAc) is a brain region that is involved in regulating behavioral responses to both natural and drug induced reward. While the NAc is mainly comprised of D1- or D2-receptor expressing medium spiny neurons (MSNs), there are several classes of GABAergic Interneurons in the area as well. MGE (medial ganglionic eminence)-
derived somatostatin (Sst) expressing interneurons account for 2-3% of the total neurons in the region and have previously been shown to be activated after cocaine administration. We show that after 7 days of I.P. cocaine Sst transcription is increased in the NAc up to 4 hours following the last injection. This supports our previously reported finding that 7 days of IP cocaine leads to a decrease in H3K27Me3 binding at the Sst promoter in NAc. We utilized optogenetics, viral mediated circuit tracing, along with stem cell transplantation of MGE interneuron precursors to study the role of NAc Sst interneuron populations in behavioral responses to cocaine. We found that NAc Sst interneurons are innervated by many of the same glutamatergic, dopaminergic, and serotonergic afferents as D1/D2 MSNs in the region and that these circuits undergo remodelling after chronic cocaine exposure. Using optogenetics \textit{in vivo} to control the activity of NAc Sst interneurons, we show that stimulation of NAc Sst interneurons suppresses locomotor responses and conditioned place preference (CPP) to cocaine, whereas silencing the interneurons has similar but distinct effects, suggesting that the activity of NAc Sst-interneurons plays a critical role in regulating cocaine induced plasticity in the NAc. We next studied the effect of transplanted fetal MGE cells in cocaine action and found that MGE transplantation into NAc, which produces an increase in the number of Sst interneurons, reduced CPP scores, suggesting that transplanted Sst-interneurons are functionally equivalent to endogenous NAc Sst interneurons. Finally, we performed RNA-seq on FACS isolated NAc Sst interneurons after chronic cocaine administration \textit{in vivo} and identified genome wide transcriptional changes induced by cocaine specifically in this cell type. By combining molecular and behavioral analyses of this specific cell type, we hope to contribute to the fields of addiction and stem cell research to ultimately gain a deeper understanding of the fundamental molecular, electrophysiological, and epigenetic mechanisms that regulate cocaine-induced neuroplasticity.

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E.A. Ribeiro: None.  
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Poster  
506. Cocaine: Reward, Sensitization, and Locomotion

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIDA
Cocaine regulates monoubiquitination of histones H2A and H2B in nucleus accumbens

Abstract: Although the etiology of drug addiction is multi-factorial, mounting evidence suggests that drug-induced alterations in gene expression in the brain's reward circuitry contribute to the chronic, relapsing nature of the syndrome. Our group and others have shown that histone post-translational modifications represent one important mechanism by which chronic exposure to cocaine induces these changes. Histone ubiquitination, despite being known to exist for decades, is less well studied than other histone marks. The dominant form of ubiquitinated histones in the cell are monoubiquitinated H2A and H2B, and both have been shown to regulate transcription in yeast and cultured human cells. However, very little is known about the role of histone ubiquitination in brain, especially in disease models such as drug addiction. Here, we show that repeated cocaine administration in mice and cocaine self-administration in rats regulate levels of H2A and H2B monoubiquitin as well as levels of monoubiquitin ‘writer’ and ‘eraser’ enzymes in the nucleus accumbens, a key brain reward region. Quantitative ChIP is currently being performed to identify how these marks are distributed at genes known to be regulated by cocaine. We are additionally examining the effect of histone monoubiquitin on behavioral responses to cocaine by viral-mediated overexpression or knockdown of H2A ubiquitin ligases Ring1 and RNF2. To our knowledge, this is the first evidence for a role of histone ubiquitination in the pathophysiology of drug addiction and points to a new area of research with potential therapeutic benefits.


Abstract: Dendritic spines are the sites of most excitatory synapses in the central nervous system, and withdrawal from drugs of abuse alters the density and morphology of dendritic spines on medium spiny neurons (MSNs) of the nucleus accumbens (NAc), a primary reward region. Members of the Rho subfamily of Ras-like small GTPases are critical regulators of spine morphogenesis in MSNs, and guanine nucleotide exchange factors (GEFs) directly activate small GTPases. Our studies indicate that early withdrawal from both investigator-administered and self-administered cocaine increases the synaptic expression of the Rap1 small GTPase in the NAc. Conversely, late withdrawal from cocaine decreases synaptic NAc Rap1 levels. To date, no downstream effectors of Rap1 in NAc MSNs have been identified, and here we characterize a novel role for Rap1 in stimulating the activity of an AKT/mammalian target of rapamycin (mTOR) local translation network within dendritic spines. Via viral-mediated gene transfer and pharmacological manipulations, we found that altered Rap1-AKT-mTOR signaling controls NAc spine morphogenesis with resulting time-dependent effects on cocaine-mediated behavioral reward. Using optogenetic methods we dissected the excitatory inputs to the NAc that regulate Rap1-AKT-mTOR signaling. These optogenetic studies revealed a specific role for prefrontal cortex (PFC) to NAc projections in increasing synaptic Rap1-AKT-mTOR activity, and we found that PFC terminal stimulation in the NAc increases behavioral reward through Rap1. Our recent work has identified specific proteins that are locally synthesized in NAc synaptosomal fractions through mTOR, and current studies are aimed at determining how these locally formed proteins regulate cocaine-mediated spine morphogenesis and behavioral reward. Supported by NIDA


Poster

506. Cocaine: Reward, Sensitization, and Locomotion

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 506.15/M4

Topic: C.17. Drugs of Abuse and Addiction
Title: Cocaine-induced enhancement of D1, and suppression of D2, medium spiny neuron activity in the nucleus accumbens is associated with cocaine seeking

Authors: *E. S. CALIPARI*1, R. C. BAGOT2, I. PURUSHOTHAMAN1, T. J. DAVIDSON3, S. T. PIRPINIAS4, K. GUISE1, K. DEISSEROTH4, E. J. NESTLER1; 1Mount Sinai Sch. of Med., New York, NY; 2Neurosci., Icahn Sch. of Med. at Mount Sinai, New York, NY; 3Dept. of Bioengineering, Stanford Sch. of Med., Palo Alto, CA; 4Bioengineering; Psychiatry & Behavioral Sciences; HHMI, Stanford Univ., Stanford, CA

Abstract: Reward learning is robust and long-lasting, and cue/context presentation elicits seeking for the previously paired reward. This process is dysregulated in cocaine addiction, whereby potentiated cue/context-reward associations combined with an inability to extinguish previously learned drug associations are thought to drive relapse. Previous work has defined the role of dopaminergic neurotransmission in cue/contextual learning and outlined how these processes are altered in nucleus accumbens (NAc) following cocaine administration; however, dopamine has opposing actions at D1- versus D2-type medium spiny neurons (MSNs) in the NAc, making it critical to determine how cocaine produces lasting alterations to each of these neuronal subpopulations. Utilizing fiber photometry calcium imaging in freely moving animals, we dissected the cell-type specific mechanisms encoding cocaine reward as well as context-elicited cocaine seeking. We virally targeted the genetically encoded calcium indicator, GCaMP6f, in mice that express Cre-recombinase in D1 or D2 MSNs and recorded activity in NAc during acquisition, expression, and extinction of conditioned place preference. Subsequently, we determined how prior chronic exposure to cocaine altered these processes. Consistent with previous work, acute cocaine administration increased D1 MSN activity, while reducing D2 activity, suggesting that biasing towards D1 MSN output is a critical determinant in the ability to form context associations. Further, we found that D1 and D2 MSNs encode very different temporally specific information about associations. Enhanced D1 MSN activity immediately preceded entry into the drug-paired context, while D2 activity was suppressed only after entry into this context. Both D1 and D2 effects diminished across extinction of place preference. Chronic cocaine administration facilitated place preference and impaired extinction, effects which were positively correlated with D1 activity, suggesting that cocaine experience strengthens context/cue-reward associations via augmented D1 firing. Together, we demonstrate distinct temporal patterns of D1 and D2 MSN signaling in the NAc associated with cocaine reward learning, providing new insight into the circuit basis of drug-cue associations and drug
Activation of estrogen receptors in the nucleus accumbens enhances the development of cocaine conditioned place preference in female mice

Abstract: Clinical studies indicate that there are sex differences in behavioral responses to cocaine, with females being more sensitive than males. Findings from humans and animals suggest that estradiol might contribute to these differences. The aim of this study was to identify the specific estrogen receptor (ER) that modulates the rewarding properties of cocaine and to investigate the molecular mechanisms responsible for this effect. Ovariectomized female mice were treated with specific agonists to ERα or ERβ in the cocaine conditioned place preference (CPP) protocol. Mice underwent 6 conditioning sessions (3 with intraperitoneal injections of 5 mg/kg cocaine and 3 with saline). ER agonists were administered 1 hour before each conditioning session. We found that the ERβ agonist, diarylpropionitrile (DPN; 1 and 5mg/kg) significantly increased cocaine CPP compared to a vehicle-treated group. Activation of ERα by propylpyrazoletriol (PPT; 1mg/kg) also resulted in a slight increase in cocaine CPP, but this increase was not statistically significant. To complement these findings, we stereotaxically injected lentiviral vectors that express short hairpin RNAs (shRNAs) targeting ERα or ERβ in the nucleus accumbens of intact female mice. Mice injected with lentivirus expressing either ERα or ERβ shRNAs exhibited decreased cocaine CPP compared to mice injected with lentivirus expressing a control non-targeting shRNA. Together, our results suggest that activation of both ERα and ERβ might play a role in the enhancement of cocaine reward in females.
Abstract: Drug memories that associate contextual cues with the effects of drugs are known to shape persistent drug seeking behaviors. Since the transfer of glycogen-derived lactate from astrocytes to neurons is required for long-term memory (Suzuki et al., 2011), we explored the possibility that disrupting glycogenolysis in the basolateral amygdala (BLA) could impair the acquisition and maintenance of positive affective memories associated with cocaine-associated cues. We have observed that rats that received intra-BLA infusions of the inhibitor of glycogen phosphorylase, 1,4-dideoxy-1,4-imino-D-arabinitol (DAB 300 pmol/side) did not acquire a cocaine-induced conditioned place preference (CPP). Most importantly, a double DAB infusion (15 minutes prior and 5 hours after contextual re-exposure) in conditioned rats exhibiting a strong preference for the cocaine compartment abolished the cocaine attractiveness for up to two weeks. Finally, we demonstrated that drug memory was rescued by L-Lactate co-administration through a mechanism requiring the synaptic plasticity related transcription factor Zif268, and extracellular signal-regulated kinase (ERK) signalling pathway. We then targeted the prefrontal cortex (PFC) with a similar protocol, but rats continued to exhibit a strong preference for the cocaine compartment. However, recent evidence established that consolidation of drug reward memories depended on successive phases (Gholizadeh et al, 2013), with the BLA involved in the early phase and the PFC possibly involved in the late phase of memory consolidation.
confirm this assumption in our model, rats were injected with DAB (480 pmol) into the PFC fifteen minutes and twelve hours after the contextual re-exposure. In contrast to rats injected with DAB 15 min/5h, those treated 15 min/12h exhibited a significantly reduced exploration of the cocaine compartment. Taken together, these results highlight a signaling role of astrocytic lactate in both acquisition and maintenance of cocaine-seeking behavior following a BLA - PFC temporal pathway.


Poster

506. Cocaine: Reward, Sensitization, and Locomotion

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 506.18/M7

Topic: C.17. Drugs of Abuse and Addiction

Support: NSERC 341673

Title: *In vivo* use of dopamine aptamers designed to cross the blood brain barrier in a preclinical mouse model of cocaine exposure

Authors: *K. VENTURA*, M. HOLAHAN, E. MCCONNELL, M. DE ROSA;
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Abstract: Presently there are few effective treatments capable of reducing the reinforcing nature or craving associated with addictive drugs. It has been well established that cocaine acts by inhibiting the reuptake of dopamine (DA); ultimately, increasing the amount of DA in the synaptic cleft. This increase in DA concentration appears to be most important for the stimulating, reinforcing, and addictive properties of cocaine. Often times, cocaine self-administration is preceded by a craving response. Studies have suggested that craving is induced by DA accumulation following cocaine addiction. Craving is also believed to be a critical factor in contributing to relapse following abstinence. This supports the need to design novel treatments capable of reducing this craving response as a treatment program for addiction. To this end, we investigated the potential use of aptamers as a novel treatment option to quell the reinforcing nature of cocaine. Aptamers are short, single-stranded DNA, RNA, or peptide sequences that exhibit unique three-dimensional structures capable of binding to a specific molecular target. We synthesized an aptamer to cross the blood brain barrier designed to inhibit synaptic DA, thereby inhibiting post synaptic activation of DA receptors. First, we administered our aptamer
systemically in a chronic manner and found that repeated aptamer treatment did not induce any toxic effects. We then tested the efficacy of our aptamer in an acute model of cocaine exposure. Animals were pre-treated systemically with the aptamer at two different concentrations then given an intraperitoneal injection of either 1, 5, or 10 mg/kg of cocaine. Locomotor activity was measured for the 30 minutes immediately following the cocaine injections. Animals were then euthanized and tissue samples were collected. As was expected, we found that cocaine increased locomotor activity in a dose-dependent manner. Importantly we found that our aptamer was able to attenuate this drug-induced behaviour with no difference between the two aptamer concentrations. It is possible that no difference was observed at different aptamer concentrations due to potential aggregation of the aptamer within the liposome that may have limited the amount of the aptamer capable of crossing the blood brain barrier. To test this hypothesis we took tissue punches from multiple brain regions and peripheral organs and analysed via qPCR to quantify the amount of aptamer present in the tissue. In summary, our DA aptamers have been shown to cross the blood brain barrier and exhibit the ability to significantly decrease cocaine-induced hyperlocomotor activity.


Poster

506. Cocaine: Reward, Sensitization, and Locomotion

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 506.19/M8

Topic: C.17. Drugs of Abuse and Addiction

Support: National Research Foundation of Korea (NFR) grant funded by the Korea government (MEST) (2014R1A2A2A01005851)

Title: Effect of acupuncture on prefronto-cortical modulation of VTA GABA neuron activity in acute cocaine-treated rats

Authors: *S. KIM¹, M. KIM², Y. FAN², B. LEE¹, Y. GWAK¹, H. KIM¹, C. YANG¹;
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Abstract: Glutamatergic neurons projecting from the prefrontal cortex to the ventral tegmental area (VTA) appear to activate VTA GABA neurons, thereby inhibiting dopamine release in the nucleus accumbens (NAc). Our previous studies have shown that acupuncture at Shenmen (HT7)
points reduced cocaine-primed reinstatement of cocaine-seeking behavior via activation of GABA neurons in the VTA. The present study was carried out to investigate the effects of HT7 acupuncture on prefronto-cortical modulation of VTA GABA neuron activity in acute cocaine-treated rats using electrophysiological methods and microdialysis. HT7 acupuncture inhibited decreases in glutamate and GABA release and GABA neuron firing rate in the VTA induced by a systemic cocaine challenge, which was reversed by GABA mixture microinjections into the infralimbic cortex, but not the prelimbic cortex. Acupuncture activated neurons in the infralimbic cortex and increased glutamate and GABA release and GABA neuron firing rate in the VTA. Similar to this result, activation of infralimbic projections by microinjection of PEPA, a positive allosteric modulator of AMPA receptors, enhanced glutamate and GABA release in the VTA. Our results suggest that glutamatergic neurons in the infralimbic cortex may mediate acupuncture’s role in modulating VTA GABA neuron activities and suppressing the reinforcing effects of cocaine.


Poster

506. Cocaine: Reward, Sensitization, and Locomotion

Location: Hall A

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Topic: C.17. Drugs of Abuse and Addiction

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Korea Institute of Oriental Medicine (K13290)

Title: Mediation of lateral habenula in acupuncture inhibition of cocaine-induced locomotor activity

Authors: *S. CHANG¹, D.-H. KIM¹, Y. RYU², Y. GWAK¹, Y. FAN¹, H. KIM¹, S. BANG¹, C. YANG¹, H. KIM¹;
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Abstract: Our previous studies have shown that acupuncture at Shenmen (HT7) points suppresses addictive behaviors of abused drugs including cocaine, alcohol and morphine and the effects are mediated by A-fiber activation of ulnar nerve originating from superficial and deep tissue. It is known that lateral habenula (LHb), an epithalamic structure, is excited by peripheral sensory stimuli and its activation produces strong inhibition of midbrain dopamine neurons thereby leading to an inhibitory influence on positive reinforcement. To explore if lateral habenula mediates the inhibitory effects of acupuncture on addictive behaviors, we tested (1) the effects of acupuncture at HT7 on cocaine-induced locomotion after electrolytic lesions of LHb or its output tract, fasciculus retroflexus. (2) excitation of LHb neurons during acupuncture stimulation by using extracellular recordings and (3) c-Fos expression in VTA-projecting LHb neurons following acupuncture by immunohistochemistry and retrograde labeling of Fluorogold. Locomotor activity was measured using a video tracking system after an intraperitoneal injection of cocaine (15 mg/kg) in male Sprague-Dawley rats. Acupuncture was applied at bilateral HT7 points for 20 s using a mechanical acupuncture device immediately after systemic cocaine injection. HT7 acupuncture suppressed cocaine-induced locomotor activity, which was blocked by lesion of either Lateral habenula or fasciculus retroflexus. An increase in action potentials recorded from extracellular recordings and c-Fos expression in LHb was seen in HT7-treated rats. These results suggest mediation of lateral habenula in inhibitory effects of acupuncture on cocaine-induced locomotor activity.


Poster

506. Cocaine: Reward, Sensitization, and Locomotion

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

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Topic: C.17. Drugs of Abuse and Addiction

Support: Pilot Grant Vanderbilt Conte Center P50 MH096972 (LDS)

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Fellowship SNF P2BSP3_148495 (LDS)

NIH award MH095277 (RDB)
Title: Molecular and behavioral contributions to cocaine action arising from SERT inhibition as studied in the SERT I172M mouse model

Authors: *L. D. SIMMLER¹, M. H. LEVIN¹, N. M. VASWANI¹, J. WANG², B. ZHANG²,³, R. D. BLAKELY¹,⁺,³; ¹Pharmacol., ²Biomed. Informatics, ³Silvio O. Conte Ctr. for Neurosci. Res., ⁴Psychiatry, Vanderbilt Univ., Nashville, TN

Abstract: Cocaine abuse remains a world-wide health problem. The psychostimulant inhibits the dopamine- (DA), serotonin- (5-HT), and norepinephrine-reuptake transporters (DAT, SERT, and NET), inducing complex molecular and circuit level plasticities that remain an active area of investigation. We developed a mouse model with an I172M substitution in the SERT gene (Slc6a4) that significantly diminishes the sensitivity of the transporter to cocaine. In these mice cocaine (20 mg/kg) fails to elevate extracellular 5-HT in vivo. Here, we describe efforts to capitalize on the SERT M172 model to 1) identify specific brain regions with SERT-dependent activation after acute cocaine administration, 2) establish transcriptional networks linked to these areas of activation and 3) evaluate contributions of SERT antagonism to the behavioral actions of repeated cocaine injections. To determine brain regions where SERT antagonism may play a prominent role in cocaine action, we quantified c-Fos positive nuclei in male and female WT and SERT M172 mice after a single 20 mg/kg i.p. cocaine injection. In these studies, we found no genotype differences in c-Fos activation in the nucleus accumbens. In contrast, cocaine-induced increase in c-Fos staining was significantly higher in the prelimbic cortex (PrL) of SERT M172 mice compared to WT, suggesting the presence of a strong serotonergic suppression of neuronal activation in the PrL of WT animals. As with c-Fos studies, RNASeq-defined transcriptome profiles of the nucleus accumbens demonstrated few differences after cocaine injections comparing SERT M172 and WT animals. In contrast, in the PrL we found genotype-dependent activation of cell signaling networks, including those linked to DA and 5-HT pathways. Finally, with repeated cocaine administration (15 mg/kg, i.p.), we assessed the contributions of serotonergic signaling to locomotor sensitization and conditioned place preference (CPP). We found no genotype effects on baseline locomotor stimulation by cocaine, nor in the overall capacity for sensitization. However, when analyzed individually, we found a significant impact of the SERT M172 mutation on the sensitization ratio (challenge vs. first cocaine application) of male mice (WT, 1.8 ± 0.2 vs SERT M172, 5.3 ± 1.4; mean ± SEM, N=12-14), suggesting a male-specific role of 5-HT in attenuating cocaine-induced behavioral plasticity. In the CPP paradigm, both male and female WT and SERT M172 mice demonstrated equivalent preference for cocaine. Our ongoing studies with the SERT I172M model offer insights into possible 5-HT contributions to the adaptations that underlie stress-induced cocaine reinstatement.

Title: GIRK channels in VTA DA neurons regulate the sensitivity of the mesolimbic DA system to cocaine

Authors: *L. A. KOTECKI	extsuperscript{1}, N. M. MCCALL	extsuperscript{2}, N. C. VICTORIA	extsuperscript{1}, N. CARLBLOM	extsuperscript{1}, K. WICKMAN	extsuperscript{1};

	extsuperscript{1}Pharmacol., \textsuperscript{2}Neurosci., Univ. of Minnesota, Minneapolis, MN

Abstract: The ventral tegmental area (VTA) is a key anatomic substrate for reward, for both naturally reinforcing stimuli and drugs of abuse. The VTA is a heterogeneous nucleus consisting of dopamine (DA), GABA, and glutamate neurons. DA neurons are the most abundant neuron population in the VTA, and they mediate the increase in DA neurotransmission in the mesolimbic DA system triggered by \textit{in vivo} exposure to drugs of abuse. As the loss of GIRK channel activity in projection targets of the VTA, such as the mPFC, are also known to show enhanced behavioral sensitization to cocaine, here we evaluated the impact of the loss of GIRK channels in VTA DA neurons on reward-related behaviors triggered by cocaine. Our preliminary data using a novel conditional mouse line where \textit{Girk2} was ablated in DA neurons (DATCre:\textit{Girk2}\textsuperscript{flox/flox} mice) show that loss of GIRK2 blunts the GABA\textsubscript{A}R-dependent inhibition of VTA DA neurons. In these studies we also show that GIRK2 ablation leads to loss of autoreceptor-dependent inhibition in VTA DA neurons. This correlates with enhanced locomotor responses to acute and repeated cocaine relative to control mice. Moreover, data from conditioned place preference and other reward-related assays in DATCre(+)::\textit{Girk2}\textsuperscript{flox/flox} mice, along with regional and cell-type specific DREADD manipulations, suggest that GIRK channel activity in VTA DA neurons controls the sensitivity of the mesocorticolimbic system to cocaine.

Ablation of the patch compartment reduces cocaine-induced stereotypy

Repeated exposure to cocaine (COC) induces stereotypy, which is characterized as inflexible, repetitive behavior. Enhanced relative activation of the patch compartment of the striatum has been shown to positively correlate with the emergence of stereotypy following repeated COC treatment, suggesting that stereotypy may be related to preferential activation of this region. However, the specific contribution of the patch compartment to COC-induced stereotypy following repeated exposure is unknown. To elucidate the involvement of the patch compartment to the development of stereotypy in response to repeated COC exposure, we determined if destruction of this sub-region altered COC-induced behaviors. Animals were bilaterally infused in the striatum with the neurotoxin dermorphin-saporin (DERM-SAP; 17 ng/µl) to ablate the neurons of the patch compartment and allowed to recover for eight days. The animals were given daily injections of COC (25 mg/kg) or saline for one week, followed by a weeklong drug-free period. Animals were then given a challenge dose of COC, placed in activity chambers, observed for 2h and sacrificed. DERM-SAP pretreatment reduced the number of mulabeled patches in the striatum. DERM-SAP pretreatment significantly reduced the intensity and spatial immobility of COC-induced stereotypy. In support of this observation, increased locomotor activity was seen in DERM-SAP pretreated, COC-treated animals. DERM-SAP pretreatment attenuated COC-induced c-Fos expression in the patch compartment, while enhancing COC-induced c-Fos expression in the matrix compartment. These data indicate that the patch compartment is necessary for repetitive behavior and suggests that alterations in activity in the patch vs matrix compartments may contribute to this phenomenon.

Title: Contribution of stress to the effects of a 5-HT1B receptor agonist on cocaine-induced locomotion before and after abstinence from repeated injections in C57BL/6 mice

Authors: *T. DER-GHAZARIAN1, K. DAI2, S. BRUNWASSER2, R. GARCIA2, K. STEFANKO2, N. PENTKOWSKI2, J. NEISEWANDER2; 1Sch. of Life Sci., 2Arizona State Univ., Tempe, AZ

Abstract: We previously showed that 5-HT1B receptors (5-HT1BRs) modulate cocaine abuse-related behavior in opposite directions depending on the addiction cycle phase (i.e., maintenance vs. abstinence) in rats. Recently we showed that C57BL/6 mice treated daily with either saline (1 mL/kg, IP) or cocaine (15 mg/kg, IP) for 21 days exhibited different responses to test day pretreatment with the 5-HT1BR agonist CP94253 (CP) depending on whether the agonist was given on the last day of the chronic treatment or 21 days later. Specifically, CP increased locomotion on the last chronic treatment day but decreased locomotion when given 21 days later, and surprisingly this effect occurred regardless of whether the mice had received chronic cocaine or chronic saline. In this study we assessed whether the flip in the agonist effect on locomotion in chronic saline-treated mice was due to injection stress and/or housing with chronic cocaine-treated mice. Drug-naïve mice arrived at the same age as mice from our previous study. One group received NO INJECTIONS and were handled twice per week during which their tails were colored for identification. Another group was treated similarly except that they received daily repeated SALINE INJECTIONS at the same time of day for 21 days similar to our previous study. On the last day of treatment, after a 1-h habituation period in test chambers, mice in both groups received either vehicle (1 mL/kg, IP) or CP (10 mg/kg, IP) and were returned to their home cage for 30 min. Next, mice were injected with saline or cocaine (5 mg/kg, IP) and placed immediately into test chambers for 1 h. The same test session was repeated after a 21-day period during which mice were handled twice per week to remark tails. Our results show that drug-naïve, No Injection mice, showed no change in locomotion in response to CP on either test day. However, chronic Saline Injection mice still exhibited a mild decrease in locomotion when pretreated with CP after a 21-day abstinence period. Literature suggests that chronic injections are a stressor in mice. We postulate that the decrease in locomotion in response to CP 21 days after ending chronic Saline Injections (present study) or chronic cocaine injections (previous
study) likely involves the same mechanisms as reflected by cross sensitization between chronic injection stress and cocaine. The CP-induced attenuation effects of chronic saline stress and chronic cocaine suggests that 5-HT1BR agonists may have therapeutic potential for treating cocaine dependence.


Poster
507. Auditory Processing: Subcortical Circuits

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#/Poster#: 507.01/M14

Topic: D.02. Auditory System

Title: Ambient GABA release is detrimental to neurons in the deafferented cochlear nucleus

Authors: *B. J. CARROLL, R. L. HYSON; Florida State Univ., Tallahassee, FL

Abstract: To better understand the effects of deafness, this project examines how hearing loss disrupts the balance between excitatory and inhibitory inputs to permanently alter the auditory system. In the avian primary auditory nucleus, nucleus magnocellularis (NM), afferent deprivation resulting from deafness triggers neuronal death. While previous experiments assumed this death is due to the absence of excitatory input from the auditory nerve, we examined whether inhibitory feedback from the superior olivary nucleus might also be involved. Using an in vitro preparation, we pharmacologically altered activity at GABA receptors in NM, and assayed the treated neurons for a marker of neuronal health, antigenicity for the ribosomal antibody Y10B (Y10B-ir). Periodic application of exogenous GABA reduced Y10B-ir on the treated side of the brain slice, compared to untreated neurons on the opposite side of the same tissue section. This effect was blocked in the presence of GABA-A antagonist picrotoxin, and mimicked by periodic application of the GABA-A agonist muscimol. Additionally, whole cell voltage clamp recordings demonstrated that currents through GABA-A receptors dominate activity in deafferented NM neurons. To determine whether this ambient GABA-A activation also modulates Y10B-ir, we periodically applied the GABA-A antagonist gabazine which increased Y10B-ir on the treated side of the brain slice, compared to untreated neurons on the opposite side of the same tissue section. Our results suggest that, in the absence of excitatory input, ambient inhibition via ionotropic GABA receptors is detrimental to NM neurons. This finding implicates dysregulated superior olivary nucleus activity in deafness-induced plasticity.
Poster

**507. Auditory Processing: Subcortical Circuits**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 507.02/M15

**Topic:** D.02. Auditory System

**Title:** The ventral cochlear nucleus in humans

**Authors:** *J. S. BAIZER\(^1\), S. WITELSON\(^2\), K. WONG\(^1\);  
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**Abstract:** Auditory information is carried from the cochlea to the brainstem via the eighth cranial nerve. Afferent fibers synapse in the dorsal (DCN) and ventral (VCN) cochlear nuclei (CN). In several species, the VCN is subdivided into the anterior (VCA) and posterior (VCP) nuclei. Several classes of neurons distinguished by anatomical and electrophysiological criteria have been described in different CN subdivisions. We have recently shown, in agreement with several older studies, that both the laminar and cellular organization of the DCN in the human is unique compared to that of other mammals. By contrast, the classic studies on human VCN, using Nissl and Golgi stains, suggest that the VCN in human is relatively similar to that in other mammals, although the division into VCA and VCP has been questioned. We have examined the organization of the human VCN using the cases from the Witelson Normal Brain Collection in which we previously studied the DCN. We used Nissl staining and immunohistochemistry with antibodies to several markers to see if the subdivisions and distinct cell populations described in animals could be distinguished in humans. In agreement with earlier studies we found individual variability in the configuration of the different components of the cochlear nuclei and major differences among humans, cats and rodents. We also found that immunoreactivity to three markers, nonphosphorylated neurofilament protein (NPNFP), the calcium-binding protein calretinin (CR) and a synthetic enzyme for nitric oxide, nitric oxide synthase (nNOS) labeled cells in the VCN. Both CR-ir and NPNFP-ir were seen in caudal VCN in neurons with round or oval somata. These may correspond to the octopus cells of the VCP described in other species. More rostrally, CR-ir, NPNFP-ir and nNOS-ir was seen in neurons with round somata; these neurons were embedded in a meshwork of stained processes, probably dendrites, running in all directions. These neurons may correspond to the bushy cells of the VCA. The results suggest that
the human VCN does include two subdivisions and that at least two major neuron types
described in VCN of other animals are present in the human VCN.

Disclosures:  J.S. Baizer: None.  S. Witelson: None.  K. Wong: None.

Poster

507. Auditory Processing: Subcortical Circuits

Location:  Hall A

Time:  Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#:  507.03/M16

Topic:  D.02. Auditory System

Support:  DOD #PR081241

KIDDRC (NICHD HD02528

Madison and Lila Self Graduate Fellowship

Title:  Quantification of auditory and non-auditory input to the dorsal cochlear nucleus

Authors:  *C. NEAL\textsuperscript{1}, H. STAECKER\textsuperscript{2}, D. DURHAM\textsuperscript{2};
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Abstract:  Increasing evidence implicate the dorsal cochlear nucleus (DCN) in the induction of
chronic tinnitus, which is the perception of sound with no corresponding external stimulus.
Fusiform cells within the DCN integrate auditory and non-auditory input via a cerebellar-like
circuit. Acoustic trauma results in reduced output from the cochlea to the dorsal cochlear nucleus
due to damage and loss of the sensory hair cells (HC). This damage initiates neuroplastic
changes within the DCN, which redistributes auditory and non-auditory inputs to the fusiform
cell circuit. Differential distribution of the vesicular glutamate transporters 1 and 2 (VGlut1/2) in
the DCN allows for visualization of the auditory (VGlut1) and non-auditory input (VGlut2),
respectively. We have previously quantified HC loss resulting from exposure to two 16 kHz
sound damage paradigms (114 dB, 1 hour and 118 dB, 4 hour) in our rat model of tinnitus. The
current study aims to quantify the distribution of auditory and non-auditory input in the DCN in
control animals prior to evaluation in sound damaged animals. Rats were perfused using 4%
paraformaldehyde and brains were harvested, embedded in 10% gelatin, and sectioned (40 um)
on a vibratome. Sections were then processed for fluorescent immunohistochemistry using
antisera to VGlut1 and VGlut2 and imaged at 20X using confocal microscopy. The Otsu method
of automated local thresholding was applied. The area of our regions of interest was determined,
and puncta number was calculated, using automated counting in ImageJ. Puncta density can be
plotted as a function of DCN layer (granule, fusiform, and deep) and/or frequency region (high, mid, and low). Initial quantification of puncta density in control animals suggests auditory input (VGlut1) is evenly distributed across the deep and fusiform cell layers of the DCN. The density of non-auditory input (VGlut2) across the fusiform cell and granule cell layers is greater than that of VGlut1. Additionally, these data show VGlut2 density peaking in the mid-frequency region, with reduced density in the low and high frequency regions.


Poster

507. Auditory Processing: Subcortical Circuits

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 507.04/M17

Topic: D.02. Auditory System

Support: DFG SFB 870 A10

Title: Postnatal refinement of conduction velocity of inputs to the medial nucleus of the trapezoid body is accompanied by changes in intrinsic cell characteristics

Authors: *J. L. SINCLAIR; Div. of Neurobio., Ludwig Maximilians Univ. Munich, Munich, Germany

Abstract: The trapezoid body (TB) is a myelinated commissural pathway projecting from the ventral cochlear nuclei on one side of the brainstem to the superior olivary nuclei on the other. TB axons may be large (approx. 3µm diameter), with each fiber giving rise to a single giant calyx of Held synapse. In myelinated axons fiber diameter positively correlates to an increase in conduction velocity. However, conduction velocity also depends on neuron-glia interactions which are developmentally and activity-dependently regulated. Postsynaptic to the calyx of Held are the principal cells of the medial nucleus of the trapezoid body (MNTB) which are well known for their fast and temporally precise firing behavior, facilitated by a distinct set of voltage and ligand gated ion channels. Here we ask whether conduction velocity in the afferent fibers and intrinsic properties in MNTB neurons are co-regulated during development and whether there are species specific differences between mouse and gerbil in the maturation of this pathway. Both species are invaluable animal models for auditory neurophysiology because of the genetic tools available for mice and because of the overlapping range of low-frequency hearing between gerbils and humans. We performed \textit{in vitro} patch-clamp recordings of the MNTB in mouse and gerbil. Examination of passive cell characteristics in mouse showed a decrease in
MNTB principal cell capacitance between P9 and P27 (P9: 35.9±6.8pF, n=16, P27: 30.0±7.7pF, n=29, p = 0.01, paired t test). The capacitance of MNTB neurons in mouse and gerbil was similar at P21 (m: 32.3±7.0pF, n=24, g: 30.4±14.7pF, n=30) and P27 (g: 31.9±20.1pF, n=19). Future analyses will characterize development of tonotopic gradients of pre- and post-synaptic specializations in these models. The conduction velocity of single TB fibers was measured far from the calyx of Held by whole-cell patch clamp recording from MNTB neurons while electrically stimulating the trapezoid body fibers in two locations. One stimulating electrode was placed near the midline and the other one further on the contralateral side. The distance between the two stimulating electrodes was divided by the difference in the latency of the postsynaptic responses evoked when stimulating with the different electrodes, in order to estimate a mean axonal conduction speed. Preliminary data suggest that conduction velocity increases post hearing onset in the mouse (P9: 3.9 ± 1.4m/s (n=8) P16: 6.1 ± 2.1 m/s (n=4), P27: 7.5 ± 6.3 m/s (n=3). In the gerbil, conduction speed is similar to mouse at P16 (P16: 6.1 ± 2.1m/s (n=7). Future experiments will examine earlier and later time points in both species.

Disclosures: J.L. Sinclair: None.

Poster

507. Auditory Processing: Subcortical Circuits

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 507.05/M18

Topic: D.02. Auditory System

Support: gm103503

    gm103412

    dc007695

    dc012938

Title: Ultrastructure of the mature calyx of held revealed by serial blockface electron scanning microscopy

Authors: *D. R. JACKSON1, P. S. HOLCOMB2, B. CHEN3, T. J. DEERINCK4, L. CAMPAÑOLA5, M. H. ELLISMAN4, H. VON GERSDORFF6, G. A. SPIROU2;

1Sensory Neurosci., 2Ctr. for Neurosci., 3Sch. of Med., West Virginia Univ., Morgantown, WV; 4Natl. Ctr. for Microscopy and Imaging Res., Univ. of California at San Diego, San Diego, CA;
Abstract: The calyx of Held nerve terminal, located in the auditory brainstem, is a powerful model system for studying neurotransmission and the development of neural circuitry. However, little is known about the ultrastructure of the calyx following the onset of hearing (P10-12). In order to understand the function of the calyx of Held, the detailed organization of synaptic machinery must be thoroughly characterized. To this end, we have employed serial block-face scanning electron microscopy (SBEM), three-dimensional reconstruction, and nanoscale identification of synaptic structures to produce the first complete reconstruction of a P30 mouse calyx of Held (CH) and its associated postsynaptic partner, the principal cell of the medial nucleus of the trapezoid body (MNTB). The MNTB cell was ovoid in shape, and measured approximately 24.96 µm in diameter, with a total surface area of 2011 µm². The apposition of the CH to this cell covered 430 µm, or 21.4% of the total somatic surface. This terminal contained 286 identified synapses, resulting in a synaptic density of 0.65 synapses/µm². The calyx had a maximum thickness of 4.6 µm. We also investigated morphological features of the calyceal axon. The axonal diameter was 1.97 µm and, interestingly, the myelinated axon transitioned nearly seamlessly into the calyceal body without a discernable heminode. In addition, a myelinated collateral branch extending from the calyx body was identified, and this collateral created a medium-sized terminal (27 µm²) with 17 synapses (synaptic density of 0.62 synapses/µm²) on the dendrite of a neighboring cell. These parameters were then applied to a biophysical model in the NEURON software to simulate the physiological characteristics of the mature calyx. Taken together, these findings provide a comprehensive morphological description of an adult mouse calyx, which can be used as a comparative endpoint for developmental studies and to explore functional elements of synaptic transmission.


Poster

507. Auditory Processing: Subcortical Circuits

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 507.06/M19

Topic: D.02. Auditory System

Support: Fapesp 2008/02771-6
Title: Source of monoaminergic and CART-ergic afferents to the elementary circuitry of the acoustic startle reflex

Authors: *A. V. DA SILVA*\(^1,2\), K. R. TORRES DA SILVA\(^1\), N. O. BARIONI\(^1\), S. A. RODRIGUES\(^1,3\), C. R. PADOVANI\(^1\), R. S. BEDUSCHI\(^1\), M. S. FERREIRA\(^1\), R. GOMEZ-NIETO\(^4,5\), J. A. C. HORTA-JUNIOR\(^1\);
\(^1\)São Paulo State Univ. - UNESP, Botucatu, Brazil; \(^2\)Med. Sch., Federal Univ. of Mato Grosso do Sul, Três Lagoas, Brazil; \(^3\)Tecnology Sch. of São Paulo State, Botucatu, Brazil; \(^4\)Neurosci. Inst. of Castilla y León, Salamanca, Spain; \(^5\)Inst. de Investigación Biomédica de Salamanca (IBSAL), Salamanca, Spain

Abstract: The acoustic startle reflex (ASR) is a rapid motor reaction elicited by a sudden intense acoustic stimulus. This is an acoustic-motor reflex of the brainstem conserved across mammal’s species including man. Moreover the ASR is a defensive behavior against possible aggressive circumstances and act as an alert to unexpected events. The ASR evokes skeletal muscles responses as well as autonomic nervous system with blood pressure increase and heart rate acceleration. In the rat, the elementary neural circuitry of ASR is mediated by organ of Corti ganglion cells, the cochlear root neurons (CRN), the pontine caudal reticular nucleus (PnC) and motoneurons of the spinal cord. The ASR can be modulated by habituation, sensitization, prepulse inhibition (PPI), and fear potentiation. These modulations are mediated by the influence of neuroactive substances upon the components of the neuronal circuitry of ASR. In this work we evaluated the origin of monoaminergic (serotonin and noradrenaline) and CART-ergic afferents to CRN and PnC and behavior test (startle and PPI) after neuronal lesion in this possible afferents. Adult Female Wistar rats (n=54) were submitted to injections of neuronal tracers (retrograde and anterograde), neurotoxic and identification of neuroactive substances in brain by immunohistochemistry techniques. All experimental protocols were made according to the Ethics Committee on Animal Use (protocol: 17/08). Our results demonstrated that noradrenergic area A5 (A5) is a source of noradrenergic and CART-ergic afferents to CRN and PnC, and the dorsal raphe nucleus (DR) is an origin of serotoninergic afferents to these regions. There are reciprocal connections between DR, A5 and PnC. Furthermore, after 14 and 21 days of lesion in A5, the percentage of PPI increased within the intervals of 100 and 150 milliseconds without modification of ASR. This data suggest that A5 have a direct action on the ASR elementary circuit evidenced by its connections and modulations of PPI. Moreover, A5 and DR connections bring new insights about modulation of ASR and PPI by noradrenaline, serotonin and CART.
Title: Long-term potentiation of glycinergic inhibition in the medial superior olive of Mongolian gerbils

Authors: B. D. WINTERS¹, *N. L. GOLDING²;
¹Neurosci., ²Dept. of Neurosci., Univ. of Texas at Austin, Austin, TX

Abstract: Principal neurons of the medial superior olive (MSO) in the brainstem of mammals are a key component in the processing of binaural cues used for sound localization. They detect extremely minute timing differences between the arrivals of sounds at the 2 ears by acting as coincidence detectors. Glycinergic inhibitory inputs onto MSO neurons are coordinated in time with binaural excitation and are critical for shaping their responses. This inhibitory drive is dramatically refined after hearing onset; supernumerary inhibitory inputs are pruned and those that remain are well timed with binaural excitation and concentrated onto the soma. Despite the relevance of inhibition to the function of this important circuit, little is known about the cellular mechanisms that guide these experience-dependent refinements. To mimic coincident inhibitory input activation and suprathreshold binaural excitation in acute brain slices from Mongolian gerbils in the first week of hearing (P12-15) we combined short current injections that elicited action potentials (APs) and electrical stimulation of MSO afferents. Driving both at a physiologically relevant frequency of 200 Hz induced a robust long-term potentiation of inhibitory potentials (iLTP, 54 ±5% of baseline [BL] at 24-34 min. post induction) isolated by blocking α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors with NBQX or CNQX (15 µM). Neither the coefficient of variance (0.307 BL, 0.276 end) nor the paired pulse ratio (0.996 BL, 1.038 end) of the responses were altered by iLTP suggesting a postsynaptic mechanism. The use-dependent N-methyl D-aspartate receptor (NMDAR) antagonist MK-801 (30 µM) added to the bath blocked induction of iLTP (14 ±2% of BL) indicating that calcium influx through NMDARs is a critical mechanism underlying iLTP. Neither APs nor electrical
stimulation alone (-9 ±4% and 6 ±3% of BL respectively) were sufficient to induce iLTP. NMDAR-dependent synaptic plasticity that increases synaptic strength is well correlated with morphological changes and maintenance of connections. After hearing onset in the MSO, intracellular chloride has shifted such that inhibitory synapses no longer provide their own depolarization, yet the need for experience-dependent synaptic plasticity remains. Our data suggest a synaptic plasticity model for inhibition in MSO neurons in which glutamate, either co-released with glycine from inhibitory terminals or through spillover from adjacent excitatory inputs, binds to NMDARs and coincident AP firing relieves the Mg²⁺ block, triggering calcium influx and changes in inhibitory synaptic efficacy.

Disclosures: B.D. Winters: None. N.L. Golding: None.

Poster

507. Auditory Processing: Subcortical Circuits

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 507.08/M21

Topic: D.02. Auditory System

Support: SFB665

Title: Altered synaptic balance and impaired sound processing in auditory brainstem neurons of the fragile x mouse model

Authors: E. GARCIA-PINO¹, N. GESSELE², *U. KOCH¹;
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Abstract: Individuals with fragile X syndrome (FXS) experience a myriad of symptoms including intellectual disability, language impairments and behavioural irregularities. Sensory processing abnormalities such as auditory hyperreactivity play a causal role in the progress of these symptoms. The auditory hyperreactivity can be studied in the mouse model of FXS (Fmr1 KO). These mice show abnormal acoustic startle response and increased audiogenic seizure susceptibility. However, the physiological mechanisms underlying this elevated sensitivity to acoustic stimuli in FXS remains poorly understood. Even less is known how the auditory pathophysiology progresses during development. Here, we addressed these questions by investigating the functional development of a primary sound localization circuit in the brain, the lateral superior olive (LSO), in the Fmr1 KO mice. To this end, whole-cell recordings from LSO neurons were performed on acute brainstem slices at various developmental stages. The stimulation of inputs from the ipsilateral cochlear nucleus revealed a drastic increase of
Excitatory synaptic strength of Fmr1 KO mice at the end of the third postnatal week, which was partially due to an increment of inputs converging onto one single LSO neuron. At the same time, release probability determined by high frequency stimulation remained unchanged. Furthermore, the spontaneous excitatory postsynaptic events were elevated in frequency while the amplitude and time constants remained unaffected. In contrast, inhibitory synaptic inputs from the medial nucleus of the trapezoid body were unchanged. At the end of the third postnatal week, LSO neurons of Fmr1 KO mice also showed larger input resistance and longer action potential duration. Next, we investigated whether these changes in synaptic and membrane properties, indicating enhanced excitability of this circuit, affected the auditory processing abilities of LSO neurons. To address this, single-unit *in vivo* recordings were performed on anaesthetized adult animals. Ipsilateral stimulation with pure-tones of different frequencies revealed a significant broadening of frequency tuning in LSO neurons. Bilateral stimulation at various interaural level differences (ILDs) resulted in a shift of the ILD-function, consistent with the increased excitatory synaptic drive. Altogether, our data demonstrate that the enhancement of excitatory synaptic inputs in the LSO contributes to altered balance of excitation and inhibition in the auditory brainstem, and might play a fundamental role in the auditory hyperreactivity observed in FXS.

**Disclosures:** E. Garcia-Pino: None. N. Gessele: None. U. Koch: None.

**Poster**

507. Auditory Processing: Subcortical Circuits

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program/#Poster#: 507.09/M22

**Topic:** D.02. Auditory System

**Support:** NIDA grant 3R24DA029989-0451

NIMH grant 5SC1MH 086070-04

**Title:** Glycinergic circuitry in the Inferior Colliculus

**Authors:** *A. B. LOPEZ, S. RODRIGUEZ, S. LAVANIA, M. MIRANDA; Biol. Sci., Univ. of Texas At El Paso, El Paso, TX

**Abstract:** The inferior colliculus (IC) is considered a relay center for the integration of ascending information from various lower level auditory nuclei. Therefore a fine balance between excitation and inhibition is vital for auditory processing and perception. Unlike
glutamatergic, cholinergic and GABAergic inputs to the IC which are well described, the cytoarchitecture of glycinergic fibers still remains to be fully elucidated. Glycinergic neurons are defined by expression of glycine transporters 1 and/or 2, encoded by two different genes. In this study, we have used a transgenic mouse line expressing GFP under control of the GlyT2 promoter (kindly provided by Dr. H.U. Zeilhofer, University of Zurich) to trace specific glycinergic circuits within the IC. We carried out neuronal tracing with the retrograde tracer FluoroGold delivered in the colliculus combined with immunohistochemistry. Preliminary results identify cell bodies of glycinergic neurons in several areas of the brainstem, particularly the pons and midbrain. A detailed characterization of glycinergic inputs to the IC should expand our knowledge about the auditory inhibitory circuitry.


Poster

507. Auditory Processing: Subcortical Circuits

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 507.10/M23

Topic: D.02. Auditory System

Title: Nitric signaling in the inferior colliculus

Authors: *A. W. STAFFORD*, A. HARTMAN, J. HALL;
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Abstract: Nitric Oxide (NO) is a gaseous molecule that functions as a retrograde messenger subserviving long-term potentiation in the hippocampus where it plays a significant role in learning and memory. Activation of glutamate N-methyl-D-aspartate (NMDA) receptors stimulates NO production via the activity of nitric oxide synthase (NOS). NO is released and subsequently enhances the presynaptic release of glutamate. Staining for β nicotinamide adenine dinucleotide phosphate diaphorase (NADPH-d) as well as immunohistochemical studies have revealed the presence of NOS-labeled neurons in a number of vertebrate brain structures including the inferior colliculus (IC), an important auditory processing center. These neurons presumably produce and release NO. However, the function of NO in auditory processing at the level of the IC is not known. Here we address this issue using NADPH-d histochemistry, NO microsensors, and single-unit recording combined with microiontophoresis to investigate the role of NO in the analysis of acoustic signals by neurons in the IC of the northern leopard frog, *Rana pipiens pipiens*. Five morphologically distinct classes of NOS-labeled neurons (multipolar, bipolar-fusiform, bipolar-round, triangular and piriform) were identified in the three subdivisions
of the frog’s IC (n=8) known to be involved in auditory processing; namely, the laminar, principal and magnocellular nuclei. Utilizing a slice preparation of the frog’s IC (n=4), bath application of glutamate (10-100 mM) elicited a concentration-dependent release of NO (69.4-273.1 nM). The increase in NO generation induced by glutamate was blocked by the glutamate receptor antagonist, AP5 (200 uM). In vivo iontophoretic application of L-NAME (a NOS inhibitor) was used to evaluate the effect of NO on the sound-evoked responses of neurons (n=18) in the IC. Responses to tone bursts were either phasic (n=8) or tonic (n=10). No change in discharge pattern, best frequency, threshold, or tuning curve of either phasic or tonic units was seen during L-NAME application. In contrast, tonic, but not phasic, units always showed a decrease in stimulus-evoked firing rate during L-NAME application. For 3 tonic units tested, an NMDA-induced increase in sound-evoked discharge rate was antagonized by the co-application of L-NAME. Our data suggest a role for NO in gain control in the IC that may influence the output of neural circuits engaged in the analysis of behaviorally relevant acoustic signals, such as speech.

Disclosures: A.W. Stafford: None. A. Hartman: None. J. Hall: None.
intercollicular tegmentum medial or lateral to the ICrp (MI and LI, respectively) to see if these areas are distinguishable in guinea pigs. We assessed soma size as well as staining for glutamic acid decarboxylase (GAD, a marker of GABAergic cells), perineuronal nets (PNs), and dense axosomatic input from vesicular glutamate transporter 2 (VGLUT2) immunopositive terminals. We have shown previously that these markers distinguish the ICc, ICd, and ICle in guinea pig IC. Do they also differentiate the ICrp, MI, and LI? Transverse sections from 4 adult guinea pigs were stained for PNs using Wisteria floribunda agglutinin (WFA) labeled with a fluorescent tag. Tissue was also immunostained for GAD, VGLUT2, and neuronal nuclear protein (NeuN). Each marker was tagged with a different fluorophore to yield quadruple staining. With a Neurolucida system, NeuN staining was used to outline each soma in the top 4 µm of a section. Outlines were then coded based on expression of GAD and the presence of a PN or a perisomatic ring of VGLUT2 positive terminals (VGLUT2 ring). Mean soma profile area was largest in LI (158 µm²), followed by ICrp (138 µm²) and MI (125 µm²). ICrp and LI had similar proportions of GAD-immunopositive (GAD+) cells (29% and 27%, respectively) whereas MI had a lower proportion (14%). In each area mean soma size of GAD cells was larger than that of non-GAD cells. As in the three main IC subdivisions, four GAD cell types were distinguishable: GAD-only (lacking a PN and VGLUT2 ring), GAD-PN (surrounded by a PN but lacking a VGLUT2 ring), GAD-VGLUT2 ring (having a ring but lacking a PN), and GAD-PN-VGLUT2 ring (having both PN and ring). The 3 regions differed in their proportions of the four GAD cell types. LI contained the highest proportion of both GAD-PN and GAD-PN-VGLUT2 ring cells (16% and 28% of all GAD+ cells, respectively). MI contained the highest proportion of GAD-only cells (74% of all GAD+ cells). These results support a continued distinction between ICrp and the three main IC subdivisions, as well as between ICrp, MI, and LI. PNs and VGLUT2 rings have been emphasized in the ICc; the present results suggest that these structures also play a role in more rostral IC subdivisions.


Poster

507. Auditory Processing: Subcortical Circuits

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 507.12/M25

Topic: D.02. Auditory System

Support: NIDCD Grant 5R01DC04199

NIDCD Grant 1F30DC014177
Title: Neural circuit reorganization in the auditory midbrain of mice with behavioral evidence of tinnitus

Authors: *J. J. STURM*¹, H. ROOS¹, T. NGUYEN², K. KANDLER¹;
¹Otolaryngology, Univ. of Pittsburgh Sch. of Med., Pittsburgh, PA; ²The Col. of New Jersey, Ewing, NJ

Abstract: Hearing loss leads to a myriad of cellular and synaptic changes in the central auditory system, some of which are thought to lead to the perception of phantom sounds (tinnitus). However, the synaptic circuit mechanisms contributing to tinnitus have remained poorly understood. To identify hearing-loss-induced reorganizations of synaptic circuits that correlate with tinnitus, we applied laser-scanning photostimulation (LSPS) with caged glutamate to map the organization and strength of intrinsic excitatory and inhibitory synaptic inputs onto glutamergic and GABAergic neurons in the inferior colliculus (IC) of noise-traumatized mice. Tinnitus behavior was quantified using the acoustic startle gap detection method. One week after noise-exposure, about 50% of noise-exposed mice (19/37 animals) showed behavioral evidence of tinnitus, as evidenced by significant reductions in gap-mediated inhibition of the acoustic startle reflex. All noise-traumatized mice had similar hearing loss and exhibited substantial reorganization in IC circuits. Interestingly, however, only mice with tinnitus showed a profound shift in the balance of synaptic excitation and inhibition in both glutamergic and GABAergic neurons. Our findings suggest that in the IC, noise trauma leads to a complex yet cell-type specific reorganization of excitatory and inhibitory local circuits, the nature of which correlates with the presence or absence of behavioral evidence of tinnitus.


Poster

507. Auditory Processing: Subcortical Circuits

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 507.13/M26

Topic: D.02. Auditory System

Support: NIH DC04391

NIH DC012450

Title: Projections from the auditory midbrain to the superior colliculus and the thalamus have separate origins
Authors: *J. G. MELLOTT, B. R. SCHOFIELD;*  
Anat. and Neurobio., NEOMED, Rootstown, OH

**Abstract:** The auditory midbrain, including the inferior colliculus (IC) and the nucleus of the brachium of the IC (NBIC), are critical contributors to the analysis of sound location. Spatial information is transmitted from the IC and NBIC to both the medial geniculate body (MG) of the thalamus and the superior colliculus (SC). The MG projects to auditory cortex and thus contributes to many aspects of auditory perception. The SC is tied to orienting movements, and may contribute as well to attention. An unanswered question is whether the spatial information sent to these targets from the IC and the NBIC arises from different populations of cells or arises (in whole or in part) as branching axonal projections from a single population of cells. Separate origins would allow for different information to be sent to each target (or for that information to be modulated independently). In contrast, branching axonal projections (i.e., collaterals) would suggest similar information is transmitted and modulation would likely affect both targets. We used double labeling with the fluorescent retrograde tracers to identify individual cells in the IC or the NBIC that send branching axonal projections to both the SC and MG in adult pigmented guinea pigs. We injected one tracer (red RetroBeads [RB] or FluoroGold; [FG]) into one MG and injected the other tracer into the homolateral SC in the same animal. Both targets received large deposits of tracer in order to maximize the number of labeled cells. After 5-7 days for axonal transport, we analyzed single and double labeled cells in the IC and NBIC ipsilateral to the injections. Injections into the MG covered much of the nucleus and labeled many cells in the IC and the NBIC. Injections into the SC labeled large populations of cells in the NBIC, the rostral pole of the IC (ICrp) and the lateral cortex of the IC (IClc). Despite the overlapping distributions in the NBIC, the ICrp and the IClc, few cells were double labeled. Preliminary quantitative analysis suggests double labeled cells (in ICrp, IClc and NBIC, combined) constituted 1.6% of the 5,361 MG-projecting cells and 5.2% of the 1,614 SC-projecting cells. We conclude that the spatial (or other) information transmitted to the SC or to the MG arise from separate populations of cells in the IC and NBIC. The existence of separate origins would allow the auditory midbrain to send different information to the MG and the SC, and also allow for these two pathways to be modulated independently (even if similar information is transmitted).

**Disclosures:**  
J.G. Mellott: None.  
B.R. Schofield: None.

**Poster**

507. Auditory Processing: Subcortical Circuits

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 507.14/M27
Title: Neurons projecting from the mouse lateral cortex of the inferior colliculus to the auditory thalamus are organized into distinct clusters correlating with glutamic acid decarboxylase-positive modules

Authors: *A. M. LESICKO, D. A. LLANO; Univ. of Illinois At Urbana-Champaign, Urbana, IL

Abstract: While the inferior colliculus is classically viewed as an integration center for ascending auditory information, its lateral cortex likely performs a distinct function, as evidenced by its multimodal connectivity. In addition to auditory inputs, the lateral cortex receives information from a number of somatosensory and visual structures. Its major targets include the superior colliculus, paralaminar thalamic nuclei, and the central nucleus of the inferior colliculus. Previous retrograde and anterograde tract-tracing studies have indicated that both the input terminals and output cell bodies of the lateral cortex are distributed in discontinuous clusters. These clusters strongly resemble the patch-like neurochemical modules that have previously been described in the lateral cortex of the rat. In the present study, we sought to determine if the input and output clusters in the lateral cortex are spatially related to the underlying neurochemical modules in the mouse inferior colliculus. To label the outputs of the lateral cortex, injections of the retrograde tracer Fluorogold were placed into the paralaminar thalamic nuclei. Following a week survival period, animals were sacrificed and brains were processed for glutamic acid decarboxylase-67 immunohistochemistry. The cell bodies that project to the paralaminar thalamic nuclei were found to cluster within these GAD-positive modular zones. Combined with our previous data, these results reveal that the output cell bodies that project from the inferior colliculus to the paralaminar thalamic nuclei are coupled with the somatosensory input modules. The present study indicates that the lateral cortex exhibits connectional as well as neurochemical modularity, and that these two levels of organization are correlated.


Poster

507. Auditory Processing: Subcortical Circuits

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 507.15/M28
Neurons in layers 5 and 6b of the auditory cortex (A1) form a massive projection system, innervating all levels of the central auditory pathway in addition to brain areas outside the classic central pathway, such as the striatum and amygdala. Little is known about how descending projections modify the representation of auditory signals in subcortical brain areas in real time. We directed our focus to the inferior colliculus (IC), an auditory midbrain structure that receives a large number of layer 5/6b cortical projections, and asked how activation of these projections might affect the ongoing processing of sound. To address this, we used an optogenetic approach to activate the corticocollicular pathway while recording unit activity from the IC of head-fixed awake mice. We interleaved trials in which a 250 ms broadband noise burst was either presented alone or in combination with photoactivation of A1 neurons expressing ‘Chronos’, an ultra-fast channelrhodopsin. Activating A1 with a pulse of light matched in duration to the sound had only weak effects on IC unit activity, with less than 20% of IC recording sites being significantly enhanced by cortical stimulation, and an average increase in sound-evoked firing of only 6%. To identify more effective modes of cortical stimulation, we used a real time closed loop spike feedback optimization algorithm (Chambers et al., 2014) that modified the voltage command signal to the laser in search of activation patterns that could either enhance or suppress sound-evoked activity in the IC. When the algorithm was tasked with finding a stimulation pattern to maximally enhance IC firing, the number of responsive sites more than doubled and the mean sound-evoked firing was increased by 42%. The algorithm was also able to learn to maximally suppress IC firing (rather than enhance) in a specific sub-region of the central nucleus, suggesting that the net effect of cortical output in this midbrain region is bi-directional, producing either gain or attenuation depending on the temporal patterning of A1 stimulation. Having identified that corticocollicular activation patterns can lead to bi-directional modulation of IC firing rates, an important question becomes whether or not these enhancing or suppressive firing patterns arise naturally during purposeful behavior. To this end, our ongoing
work is focused on recording from genetically identified corticocollicular neurons while mice are engaged in active listening tasks.

**Disclosures:**  

**Poster**

**507. Auditory Processing: Subcortical Circuits**

**Location:** Hall A  
**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM  
**Program#/Poster#: 507.16/M29  
**Topic:** D.02. Auditory System

**Support:** NIH Grant F31 DC013501

**Title:** Making inhibition work for you: An electrophysiological study of chemical and photochemical stimulation of the thalamic reticular nucleus

**Authors:** *B. SLATER¹, D. LLANO²; ¹Neurosci., ²Mol. and Integrative Physiol., Univ. of Illinois, Urbana, IL

**Abstract:** The thalamic reticular nucleus (TRN) has been implicated to play a major role in a variety of brain functions, including sleep, attention, and information processing. One of the major tools to tackle the function on various nuclei in the brain is electrophysiological recordings using various stimulation paradigms to probe the inputs and outputs of any given brain region. Electrical stimulation is one of the most common ways to stimulate neurons, however when studying brain structures that are small and/or have white matter tracts passing though, it becomes difficult or impossible to separate what structures are being stimulated. The TRN is a small structure which surrounds much of the thalamus and has many of the thalamic axons passing through it, chemical stimulation, especially focal application, avoids some of these difficulties. Here we present a variety of chemical and photochemical methods to stimulate the thalamic reticular nucleus, and how robust each of these methods are in producing either action potentials or somatic depolarization. To test this, we used a mouse brain slice with the auditory portion of the thalamic reticular nucleus. Single cell electrophysiological recordings were done using whole-cell patch clamp and stimulation was done using either pressurized application of glutamate or acetylcholine or using a laser to photostimulate a variety of caged glutamate and cholinergic compounds. We used a variety of stimulation frequencies and intensities to explore the most robust stimulation parameters. We find that chemical cholinergic stimulation is more
robust than glutamatergic stimulation in generating action potentials and that varying frequency and intensity of stimulation can produce a variety of TRN spike outputs.

Disclosures: B. Slater: None. D. Llano: None.

Poster

507. Auditory Processing: Subcortical Circuits

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 507.17/M30

Topic: D.02. Auditory System

Support: NIH Grant DC004450

NIH Grant F31DC013223

Title: Synaptic contacts between auditory Golgi cells

Authors: *D. B. YAEGER\textsuperscript{1}, L. O. TRUSSELL\textsuperscript{2};
\textsuperscript{1}Physiol. and Pharmacol., \textsuperscript{2}Oregon Hearing Res. Ctr., Oregon Hlth. and Sci. Univ., Portland, OR

Abstract: In the cerebellum and in cerebellum-like circuits, axons of individual Golgi cells diverge to inhibit hundreds of granule cells. Recent results in the cerebellum have shown that Golgi cells make chemical and electrical synapses onto each other that promote synchronous firing of Golgi cells and coordinated inhibition of granule cells. We tested whether such a circuit arrangement exists in the dorsal cochlear nucleus using acute mouse brain slices. Injection of current into one Golgi cell resulted in a voltage deflection in a simultaneously recorded Golgi cell in over 90% of dual recordings, with an average coupling constant of 0.10 +/- 0.01 (n = 57). Electrical coupling showed only weak voltage-dependent rectification and electrical coupling was absent in the Cx36 knock-out mouse, indicating that coupling was mediated by connexin 36. Spikes in prejunctional Golgi cells evoked currents (spikelets) in postjunctional Golgi cells that were insensitive to blockers of fast chemical inhibition, suggesting that Golgi cells make electrical, but not chemical, synapses onto each other. Spikelets recorded in voltage clamp were composed of a brief inward current and a prolonged outward current, the charge of which exceeded that of the inward current, suggesting that spikelets have a net inhibitory effect on postjunctional Golgi cells. Indeed, in current clamp recordings, spikes in prejunctional Golgi cells reduced the probability of postjunctional Golgi cell spiking in response to brief current injections for tens of ms after the prejunctional spike. Despite the absence of chemical synapses between Golgi cells, bath application of the K+ channel blocker 4-AP evoked GABAergic and
glycinergic IPSCs in Golgi cells. In 3 of 30 paired recordings, molecular layer interneurons (MLIs) made inhibitory synapses onto Golgi cells, and IPSCs in MLI-Golgi cell pairs were completely blocked by the GABA-A receptor antagonist SR 95331 (n = 2). Thus, our results suggest that Golgi cells provide spike-mediated inhibition to each other through electrical synapses and also receive GABAergic inhibition from MLIs.

Disclosures: D.B. Yaeger: None. L.O. Trussell: None.

Poster
507. Auditory Processing: Subcortical Circuits

Location: Hall A
Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM
Program/#Poster#: 507.18/M31
Topic: D.02. Auditory System
Support: ONR grant N000141210731
Title: Mechanisms of auditory gain enhancement following acute noise trauma

Authors: *B. D. AUERBACH¹, K. E. RADZIWON¹, P. V. RODRIGUES², G.-D. CHEN¹, R. J. SALVI¹; ¹Ctr. for Hearing and Deafness, SUNY At Buffalo, Buffalo, NY; ²Dept. of Anesthesiology, Sch. of Med. and Publ. Hlth., Univ. of Wisconsin, Madison, WI

Abstract: Hearing loss often gives rise to debilitating auditory perceptual disorders like tinnitus- a phantom ringing or buzzing sensation - and hyperacusis- where moderate intensity sounds are perceived as intolerably loud or even painful. While tinnitus and hyperacusis are often triggered by cochlear damage, it is now widely believed that these conditions arise from pathological plasticity in the central auditory system. We and others have proposed that tinnitus and hyperacusis are the result of disrupted gain control in the central auditory system. Central auditory neurons can modulate their dynamic range based on the pattern and level of incoming sound, thereby allowing them to maintain a relatively stable range of activity and preserve neural coding efficiency. According to the central gain model, under pathological conditions such as acute noise trauma or long-term hearing loss, this gain modulation can result in the over-amplification of spontaneous neural activity and supra-threshold sounds, which may underlie tinnitus and hyperacusis. Indeed, human and animal studies indicate that despite the reduced cochlear output following sensorineural hearing loss, spontaneous and sound-evoked responses at higher auditory structures can be paradoxically increased. However, it is still unclear where this hyperactivity is initiated, how it propagates through the auditory system, and how it relates
to the spectral profile of hearing loss. To address these questions, we recorded auditory-evoked local field potentials (LFP) and multi-unit spike discharges from several auditory areas simultaneously and examined the effect of acute noise trauma that resulted in temporary threshold shifts of 10-20 decibels (dB SPL). While responses from the auditory midbrain and thalamus were generally decreased at all intensities, there was a robust enhancement of supra-threshold responses in the cortex. Current source density analysis demonstrated that this enhancement was primarily the result of enhanced intracortical processing. These results suggest that gain enhancement may not be passively transmitted from lower to higher levels of the auditory system, and in fact may originate within the cortex. This work not only has direct implications for our understanding of tinnitus and hyperacusis but is likely to provide insight more generally towards the mechanisms of experience-dependent plasticity and gain modulation in the auditory system.

**Disclosures:** B.D. auerbach: None. K.E. Radziwon: None. P.V. Rodrigues: None. G. Chen: None. R.J. Salvi: None.

**Poster**

**508. Auditory Perception, Cognition, and Action**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 508.01/M32

**Topic:** D.02. Auditory System

**Support:** AFOSR FA9550-12-10388

**Title:** Nonlinear resonance and plasticity as a basis for musical consonance

**Authors:** *J. KIM, E. W. LARGE;
Psychology, Univ. of Connecticut, Storrs, CT

**Abstract:** The consonance and dissonance of musical intervals is one of the oldest concepts in music theory and has been a subject of scientific inquiry from antiquity. Currently, there are two main theories of the basis for musical consonance. A dominant view of the past century has been that dissonance arises from sensory roughness produced by the beating of nearby frequency components and that consonance merely indicates a lack of roughness. Another view with a long history at least from the time of Pythagoras is that consonance is based on a harmonic relationship between sounds, which can be expressed as a simple integer ratio. Recent investigations have shown that harmonicity is a stronger factor in the perception of consonance than is roughness, especially for musically trained listeners. Here we explain the relationship
between harmonicity and consonance in terms of nonlinear resonance arising in the network of oscillatory neural populations tuned to auditory frequencies. We use a network of generic oscillators as a model of nonlinear auditory processing and show that the dynamical interactions between neural oscillators tuned to different frequency ratios match empirical findings and music-theoretical accounts of the relative consonance of musical intervals. A mathematical analysis of nonlinear resonance in oscillator networks shows how the strength and stability of nonlinear resonance varies as a function of natural frequency ratio and other model parameters. We also introduce Hebbian plasticity to the network of nonlinear oscillators and discuss its implications for the relationship between musical experience and the role harmonicity plays in the perception of consonance.

Disclosures: J. Kim: None. E.W. Large: A. Employment/Salary (full or part-time):; Circular Logic, LLC.

Poster

508. Auditory Perception, Cognition, and Action

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 508.02/M33

Topic: D.02. Auditory System

Support: Intramural research program of the NIH, NINDS

Title: Neural mechanisms of temporal prediction in naturalistic auditory stimuli

Authors: *B. MANISCALCO¹, P. ABRY³, T. HOLROYD³, B. J. HE¹;
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³Physics Dept., CNRS, Lyon, France

Abstract: Many features in both natural stimuli and brain activity exhibit power-law scaling relationships between power and temporal or spatial frequency (P \propto 1 / f^\beta). In the temporal domain, such scaling relationships manifest as autocorrelations that can be exploited for the purpose of prediction. Yet, the literature on prediction and prediction error has predominantly focused on stimulus sequences with relatively simple patterns of local and global regularities. Here, we investigate whether human observers can extract statistical regularities from stimuli with more complex and ecologically valid patterns of autocorrelation and use this information for temporal prediction, and if so, what neural mechanisms underlie this process. We presented subjects with sequences of tones whose pitch fluctuated over time. Pitch autocorrelation was parametrically modulated across trials, and sequences were chosen so as to yield a range of
values for the expected value of the final tone pitch. Subjects judged the degree of pitch autocorrelation in each sequence and rated the probability of the final tone. Crucially, subjects’ ratings of final tone probability tracked the expected value for final tone pitch, suggesting that subjects were able to extract the autocorrelation statistics in the sequence necessary for making valid predictions about upcoming tones. MEG data reveal that tone sequences elicited phase locking in an arrhythmic 1/f pattern in low frequencies (< 5 Hz). This component of the neural response also contributed to sequence prediction, as shown by its modulation by the expected value of final tone pitch. MEG sensors carrying this prediction signal exhibited topographical overlap with those whose activity tracked the tone pitch instantaneously or with history-dependence, suggesting a possible local computation of predicted pitch on the basis of the present and previous tone pitches. Distinct sensor clusters showed sensitivity to prediction error derived from sequence autocorrelation statistics vis-à-vis local changes in pitch, suggesting cortical separation of local and global prediction errors. Taken together, the results suggest that naturalistic stimuli with 1/f-type temporal fluctuations in stimulus properties modulate low-frequency arrhythmic brain activity, and that this arrhythmic brain activity mediates sequence prediction. This study also expands on previous studies on prediction and prediction error by employing stimuli whose statistical regularities are more complex and more representative of patterns found in natural stimuli.

Disclosures: B. Maniscalco: None. P. Abry: None. T. Holroyd: None. B.J. He: None.
Abstract: Interpreting the meaning of a sound depends on contextual cues, including other sensory stimuli and the internal state of a listener. The basolateral amygdala contributes to the analysis of a sound’s meaning by integrating information from several senses as well as internal state. The amygdala’s analysis of internal state has the potential to modulate the primary auditory system via its direct and indirect projections to auditory cortex. We hypothesized that even brief restraint, such as normally occurs in auditory neurophysiological studies, will alter stress levels as well as responses to sounds in the BLA. This study assesses effects of restraint on neurophysiological responses in the BLA as well as behavioral, vocal, and hormonal measures of stress. Blood corticosterone levels were measured in 10 free-moving and 10 restrained (2 hour) CBA/CaJ mice. In other mice, we measured the effect of brief restraint using a light/dark box, a marble bury test, and recorded vocalizations. Test mice were restrained for 2 hours on 3 consecutive days; control mice were placed in the test chamber without restraint. To assess effects on the auditory responses in the BLA, we recorded 160 repetitions of noise-evoked local field potentials (LFPs) in 13 adult male mice. Auditory responses were recorded in two sets from free moving mice, followed immediately by two sets of recordings from the same animals during restraint. Signals were transmitted wirelessly from custom multi-electrode implants using a wireless headstage. Several measures of stress were significantly higher in mice after restraint: corticosterone levels (F (19) = 10, p = 0.006), time in dark (p = 0.002), and number of buried marbles (t (15) = 3.809, p = 0.002). The frequency and duration of emitted syllables differed dramatically for mice during restraint compared to mating encounters or isolation in a novel chamber (p<0.001). The magnitude of noise-evoked LFPs was typically greater during restraint (9/13 mice, p < 0.001). A one way ANOVA revealed a main effect of discriminatory information from comparisons of recording sets (F(3,37)= 4.7, p = 0.007); the free vs. restrained comparison had significantly higher levels of discriminatory information (p<0.05). The recording condition could be determined by computations on the single trial LFP for an average of 72% of trials. Restraint comparable to that commonly used in auditory neurophysiology caused consistent changes in the neural responses to sound and generated long-lasting stress in mice. This increased neural response may contribute to the increased auditory sensitivity and improved auditory signal detection that is caused by stress.


Poster

508. Auditory Perception, Cognition, and Action

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 508.04/M35
Title: Synaptic signature of optimal and suboptimal states for sensory signal detection

Authors: *M. J. MCGINLEY¹, S. V. DAVID², D. A. MCCORMICK¹;  
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Abstract: The cortical subthreshold membrane potential and synaptic dynamics underlying optimal sensory signal detection are not well known. Human and animal studies have reported that performance on signal detection tasks is highly state-dependent, exhibiting an inverted-U dependence on arousal and the activity of neuromodulatory pathways. This relationship, known as the Yerkes-Dodson curve, predicts that optimal performance occurs at intermediate levels of arousal (Yerkes and Dodson, 1908). But what are the synaptic and circuit mechanisms of this inverted-U dependence of optimal states for behavior and neural responses? To address this question, we recorded membrane potentials of auditory cortical neurons in mice trained on a challenging tone-in-noise detection task while assessing arousal with simultaneous pupillometry and hippocampal recordings. We find that the mouse’s internal state fluctuates continuously and rapidly (in seconds or less), and arousal can be quantified simply as the diameter of the pupil. The pupil diameter closely tracks the rate of occurrence of hippocampal sharp waves. In addition, auditory cortical membrane potentials of layer 4 and 5 excitatory neurons exhibit: slowly fluctuating (1-10 Hz) rhythmic activity with low arousal; hyperpolarization and low variability at intermediate arousal; depolarization and variability with sustained hyper-arousal (with or without walking); and transient depolarization in synchrony with micro-arousal events. Optimal signal detection behavior and sound-evoked responses, at both sub-threshold and spiking levels, occurred at intermediate arousal when pre-decision membrane potentials were stably hyperpolarized. These results reveal a cortical physiological signature of the classically-observed inverted-U relationship between task performance and arousal, and that optimal detection exhibits enhanced sensory-evoked responses and reduced background synaptic activity. Furthermore, these results provide a framework with which to resolve apparent discrepancies between species and sensory systems in cortical membrane potential dynamics. Revealing the neural mechanisms by which the state of the brain and periphery is controlled on a moment-to-moment basis promises to clarify many interesting aspects of neural network function, including the neural basis of optimal performance, and may reveal a nervous system that is considerably more accurate and less variable than previously thought. Yerkes RM & Dodson DJ (1908). The relation of strength of stimulus to rapidity of habit formation. Journal of Comparative Neurology and Psychology 18, 459-82.
Poster

508. Auditory Perception, Cognition, and Action

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 508.05/M36

Topic: D.02. Auditory System

Support: Howard Hughes Medical Institute

Title: Long-lasting recalibration to auditory listening conditions

Authors: *N. C. RABINOWITZ\(^{1,2}\), M. SCHEMITSCH\(^1\), O. BRIMJOIN\(^3\), E. P. SIMONCELLI\(^{1,2}\);
\(^1\)Ctr. for Neural Sci., NYU, New York, NY; \(^2\)Howard Hughes Med. Inst., New York, NY; \(^3\)MRC Inst. of Hearing Res., Glasgow, United Kingdom

Abstract: Perception can be described as a process of inferring the properties of objects in the world from incoming sensory signals [Alhazen 1040, Helmholtz 1867, Kersten et al 2004]. This inverse process is particularly difficult as object properties are entangled with those of contextual “viewing conditions”. For example, before emitted sounds reach the ears, they undergo spectro- and spatio-temporal filtering by the room and head. The details of these transformations depend on the complex geometry and physics of a given environment, and can change considerably with head position. Thus, in order to make inferences about source objects, the brain must separate an incoming signal into "source" and "listening condition" components. We hypothesized that the brain accomplishes this feat by factoring out systematic regularities that are learned from experience. To test this, we built a novel psychoacoustic chamber, wherein features of sound sources were systematically varied according to human subjects’ head positions. When we coupled the carrier-frequency of a pure tone to subjects’ head angle, they passively learn to compensate for this in perceptual judgments, without being aware of the perturbations. Curiously, this does not occur for other sound features such as modulation rate or depth [Rabinowitz et al, ARO 2014]. Based on these findings, we developed a model for perceptual recalibration based on known physiological properties of the peripheral auditory system, and used it to predict how listeners would be biased by altered environmental statistics. We hypothesized that spectral recalibration involves a rescaling of the gain of peripheral frequency channels to partially compensate for systematic discrepancies across head angles, and that these gain changes have a fundamental resolution limit imposed by the peripheral representation. From this model, we predicted that listeners would not only compensate for spectral discrepancies that...
they had been exposed to, but that they would maladaptively “anti-compensate” at nearby frequencies which were not part of the exposure regime. Indeed, we find that this effect manifests precisely as predicted. Moreover, the spectral resolution of perceptual recalibration appears to be comparable to that of peripheral critical bands. The effects we report last for tens of minutes after the end of the exposure period. Control experiments in the closed-field rule out the possibility that they result from peripheral adaptation. Thus listeners’ inferences about sound sources can be biased by systematic relationships experienced over extended time scales, but only through means allowed by physiology.

Disclosures: N.C. Rabinowitz: None. M. Schemitsch: None. O. Brimijoin: None. E.P. Simoncelli: None.

Poster

508. Auditory Perception, Cognition, and Action

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 508.06/M37

Topic: D.02. Auditory System

Title: Measurement of acoustic frequency discrimination thresholds in common marmosets (Callithrix jacchus)

Authors: *Y. GUO, M. S. OSMANSKI, X. SONG, X. WANG;
          Johns Hopkins Univ., Baltimore, MD

Abstract: The common marmoset (callithrix jacchus), a New World non-human primate, has emerged as a promising animal model system in auditory neuroscience research. It is thus important to characterize the fundamental auditory perceptual abilities of this species, such as their capacity for frequency discrimination. We trained marmosets on a discrimination task using operant conditioning procedures and measured the minimum change in frequency (i.e., the frequency difference limen, or FDL) that marmosets could detect using pure tones at eight different frequencies. Stimuli spanned the entire hearing range of the marmoset from 220 Hz to 28.16 kHz with octave intervals. All stimuli were presented at a relatively constant sensation level. Sound level was also roved +/- 3dB to eliminate the possibility of using intensity fluctuation as a cue to perform the task. Our data show that (1) the absolute FDL increases as the testing frequency increases; (2) the relative FDL decreases from ~2.5 semitones (1 semitone equals ~6% of the testing frequency) until it reaches minimum value of ~0.5 semitones around 7kHz, then slightly increases as the testing frequency goes higher. The frequency range with lowest relative FDL overlaps with the most sensitive region in the marmoset audiogram, as well
as with the fundamental frequency range of their typical vocalizations. We also tested marmosets’ pitch discrimination abilities using harmonic complex tones with fundamental frequencies of 110 Hz, 220 Hz, 440 Hz and 880 Hz. It is shown that as the fundamental frequency increases from low to high, the relative difference limen first decreases and then increases, with a highest value of ~1.4 semitones at 110 Hz and a lowest value of ~0.4 semitones at 440 Hz. These results reveal auditory perceptual capacities of the marmoset and help guide further studies of auditory behaviors of this species. This research is supported by an NIH Grant (DC003180).

Disclosures: Y. Guo: None. M.S. Osmanski: None. X. Song: None. X. Wang: None.

Poster

508. Auditory Perception, Cognition, and Action

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 508.07/M38

Topic: D.02. Auditory System

Title: Musical interpretation in blind pediatric subjects. An MR study

Authors: *B. DE CELIS ALONSO¹, S. HIDALGO TOBÓN², P. DIES SUAREZ², C. GUERRERO ARENAS³, E. CASTRO-SIERRA³; ¹BUAP (Benemérita Univ. Autónoma de Puebla), Puebla, Mexico; ²Lab. de Psicoacústica y Fisiología Auditiva, ³Hosp. Infantil, Federico Gómez, Mexico DF, Mexico; ⁴Escuela Nacional de Musica, UNAM, Mexico DF, Mexico

Abstract: Introduction. The objective of this study was to assess the physiological differences in brain recruitment and connectivity when interpreting melodic and non-melodic music in control and blind pediatric populations. To this end, a BOLD-fMRI experiment was performed to quantify BOLD representations and connectivity. Methods. 26 pediatric subjects were recruited for this study. During the BOLD-fMRI study two stimuli were delivered to subjects: A melodic (M) and a non-melodic (N) music sequence. Eight contrasts were built: M for blind (B), N for B, M for Controls (C), and N for C. Also Blind M vs. N, Control M vs. N, Music B vs. C, Noise B vs. C. Finally, connectivity calculations were performed for these contrasts in which ROI to ROI correlations were calculated. Results & Discussion. In the BOLD study (Figure 1), cerebellum and fusiform regions played a big role in sound interpretation for both groups. Frontal lobe activations were strongly associated with music while temporal activations were found for all the sound stimuli. Regions of activation in the frontal and temporal lobes changed with the group and kind of stimuli applied. Blind volunteers had right hemisphere predominance contrasting
with the left prominence of controls. For the connectivity study (Figure 2) different networks were involved in the musical or the noise interpretation depending on the group studied. In contrast similar mechanisms existed between blind and controls when differentiating melodic from non-melodic stimuli.
Poster

508. Auditory Perception, Cognition, and Action

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 508.08/M39

Topic: D.02. Auditory System

Title: Is there an effect of long-lasting exposure to industrial noise in the adult auditory system of rats

Authors: *B. GOURÉVITCH, F. OCCELLI, J.-M. EDELIN;

Abstract: Over the last decades, an increasing number of people have been exposed on a daily basis to important levels of noise. Short term damages induced by traumatic noise (>105 dB
SPL) have been widely studied in the literature. Recent studies have shown that, even at a non-traumatic and legally authorized intensities (<85dB SPL), alterations of the auditory system occurred at the cortical (Noreña et al 2006) and peripheral levels in the absence of efferent feedback (Maison et al 2013). Only a few articles have tried to compare electrophysiology data and behavioral deficits after non-traumatic noise exposure after a 2-4 months exposure. Our whole project aims at evaluating the impact on the auditory system of adult rats after a long-lasting exposure (3-18month, starting at 2 months old) to a structured (industrial) non-traumatic noise (80dB SPL, 8h/day). Here, we assess on every single animal the consequences of such exposure on brainstem, behavioral and cortical response to noisy backgrounds and auditory contrasts. First, although auditory brainstem thresholds were affected by aging, they were not degraded by long-term exposure. Second, after very long-term exposure, exposed animals showed as good or even better response features than control animals in the primary auditory cortex. If confirmed, these results might indicate that the type of noise (pure tone vs. dynamic noise) and/or the age at which animals started to be exposed are crucial factors that influence the impact of environmental noise.

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**Poster**

**508. Auditory Perception, Cognition, and Action**

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**Topic:** D.02. Auditory System

**Support:** NIH Grant No. 1RO1 DC 006243

UTHSCSA School of Medicine Bridge Funding OSP Award 10006523

**Title:** Flexible scripted software system for delivery and analysis of F0 perturbation experiments

**Authors:** *B. ROGERS*¹, C. L. CHAN², A. B. NEW¹, C. R. LARSON², D. A. ROBIN¹;

¹Res. Imaging Inst., Univ. of Texas Hlth. Sci. Ctr., San Antonio, TX; ²Northwestern Univ., Evanston, IL

**Abstract:** The ability to control voice fundamental frequency (F0) is essential for successful and efficient speech production. Pitch perturbation techniques have often been used investigate the role of auditory feedback in F0 control. This set of four computer applications combines
Experiment scripting, paradigm presentation with real-time audio feedback, browsing of results and speech analysis. Script Generator is a web based application used to build a pitch perturbation experiment. An experiment is a set of trials with a single vocalization per trial. Script Generator provides the user with a point and click interface with options to create a sequence of events presented during a trial. Each event has a set of attributes specifying cuing, timing, vocal perturbations, and recording. Cues can be audio clips, text, or images. The timing and the magnitude of each of these events can be specified as well as the number of repetitions. A number of different trial types can be combined in an experiment. The resulting script is a JavaScript Object Notation (json) file that is then fed into the PitchPresent program. In PitchPresent, participants vocalize in response to audio or visual cues. During vocalization participants receive audio feedback where the F0 may be intermittently shifted in pitch. Headphones are worn for the delivery of audio cues and for receiving audio feedback. Visual cues to the participants are displayed on a monitor. Vocalizations are captured and saved for further analysis. The presentation of cues and the audio processing is completely software based and requires no special hardware. PitchBrowse is used for review and export of the data collected in PitchPresent. It can display audio waveforms for each trial, play the audio, and show the timing of the events during individual trials. This data can be exported in json, text, or WAV files, and in a binary format suitable for Matlab. The Speech Analysis toolkit is a web based app that parses the json data exported from the PitchBrowse program and provides the experimenter with a set of individual, averaged and waterfall graphs of the pitch contours from collected data. The participant’s response for each condition is automatically averaged and measured. All of the data are stored in a SQL database and each data set is attached to the participant’s personal and demographic information (collected during the pre-experiment stage). The results are averaged both within-participant and across participants. The four applications were developed using free or open source software. The web based applications depend heavily on Javascript. PitchPresent and PitchBrowse are written in C++ for performance reasons.


Poster

508. Auditory Perception, Cognition, and Action

Location: Hall A

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Topic: D.02. Auditory System

Support: Nasjonalforeningen for folkehelse 2014
Effects of poor hearing acuity on gait during dual-task execution: data from cognitively healthy older adults

Authors: *C. RODRIGUEZ-ARANDA, M. MITTNER, K. WATERLOO, O. VASYLENKO, M. M. GORECKA; Dept. of Psychology, Univ. of Tromsø, Tromsø, Norway

Abstract: Hearing acuity can become seriously compromised with advanced age. Troubles associated with impaired hearing include difficulties with communication, balance and mobility. In fact, reduced hearing acuity in the elderly is associated with slower walking speed and lower walking endurance. Also, hearing impairments interfere with the ability to filter out competing sound sources, which jeopardize selective attention. Clinical data suggest that a decline on hearing acuity may be a central cause of greater risk of falls in older adults who need to cope with daily situations demanding sustained attention during walking. However, to date there are no experimental data on this matter.

Aim. We investigated whether older adults with poor hearing acuity are more prone to show gait disturbances than elderly with good hearing function when performing a dual-task during walking.

Method. 29 elderly with reduced hearing acuity (M age =74.4 years) and 35 elderly with normal hearing (M age = 68.5 years) were the participants. All were right-handed and free of cognitive dysfunction. Cognitive assessment and pure tone audiometry were applied. Also, pure tone average (PTA) at thresholds 0.5, 1, 2, and 4 kHz was calculated. Best-ear PTA was used to classify groups into low (LHA) or normal hearing acuity (NHA). Participants were excluded if interaural asymmetry between ears >5dB or PTA threshold >45dB. The dichotic listening (DL) test was used as concomitant task to walking. The dual-task situation followed the 3 standard conditions of DL: Non-forced, Forced-Right and Forced-Left. Gait was evaluated over ground with the OptoGait system during dual-task performance and also during comfortable walking. Standard spatio-temporal measurements of gait were quantified.

Results. At baseline, M for step, stride, speed and distance were significantly reduced in the LHA group while CV for speed and cadence were significantly higher. Across DL conditions, double support time significantly increased in the LHA group. Especially, the LHA group showed more gait disturbances during Forced-Left condition in which M for step length and CV for step width showed asymmetric perturbations on the right foot.

Conclusions. Older adults with low hearing acuity show clear differences in spatio-temporal parameters during walking as compared to elderly with normal hearing. Moreover, LHA showed further alterations in gait stability during dual-task performance, which confirms that LHA elderly are at higher risk of falling.

Poster

508. Auditory Perception, Cognition, and Action

Location: Hall A

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Topic: D.02. Auditory System

Support: NINDS Grant 2R37NS21135

Nielsen Corporation

Title: Task-specific electrophysiological differences between musical pitch and valence judgment

Authors: *F. FOO, J. HIROTA, R. MALZYNER, R. T. KNIGHT;
Univ. of California, Berkeley, CA

Abstract: The processing of successive pitch changes and simultaneous pitch combinations are important yet distinct percepts in music, respectively facilitating the perception of musical contour and driving emotional responses toward music. The neural correlates of each percept have been studied extensively in humans, but no study to date has investigated these percepts with the same stimuli to examine task-specific differences in the cognitive processes involved. We present a novel experimental design where changes in pitch (up/down) and valence (consonance/dissonance) are both present in the same stimulus sequence. Subjects (n=22) heard 2 piano chords in succession, and made either a pitch direction judgment (Pitch Task, PT) or a valence judgment (Valence Task, VT) while EEG and behavioral data were recorded. We observed that: 1) The target chord generated a larger N1 ERP amplitude (p<0.05) that was maximal over central regions than the cue chord in PT, but no significance was observed in VT. This supports the hypothesis that pitch direction judgment requires greater attention to the target chord, while valence judgment requires a similar amount of attention to both chords. 2) Consonant chords elicited a larger P2 ERP amplitude (p<0.05) over frontocentral regions than dissonant chords in VT, but no significance was observed in PT. Notably, spectral analyses revealed greater frontal alpha asymmetries and beta reductions for VT. This suggests that attention to valence enhances the contrast between ERP and spectral responses for consonant and dissonant chords. 3) The target chord evoked a smaller P3b component (p<0.05) over centroparietal regions in VT, which may reflect higher cognitive workload and reduced decision certainty. This is further supported by increased frontocentral theta activity in VT, longer mean reaction times (PT: 1068ms, VT: 1150ms) and a negative correlation between accuracy and reaction time for VT that is absent in PT (r^2(PT): 0.007, r^2(VT): 0.342). No difference was
found in mean accuracy for either task (PT: 90.2%, VT: 91.7%). 4) A larger negative ERP component 400-500ms after stimulus onset (p<0.05) was observed for dissonant chords over frontocentral regions in PT than in VT, but this component was not present for consonant chords in either task. This is in line with findings of a similar N4 component in response to unexpected words in language tasks, and suggests a heightened unexpected quality of dissonant chords when attention was directed away from its valence. In conclusion, attention to pitch or valence differentially modulates ERP and spectral components associated with early auditory and late cognitive processing of musical percepts.

Disclosures:  F. Foo: None. J. Hirota: None. R. Malzyner: None. R.T. Knight: None.

Poster

508. Auditory Perception, Cognition, and Action

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 508.12/M43

Topic: D.02. Auditory System

Support: NEI-NIH

NIDCD-NIH

Boucai Hearing Restoration Fund

Title: Dissociation between spiking activity and local field potentials in the auditory cortex during auditory decision-making

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Abstract: Auditory decision-making requires the conversion of incoming auditory information into a categorical judgment. For a decision regarding the frequency content of an auditory stimulus, spiking neural activity in the middle-lateral belt region (ML) and the anterolateral belt region (AL) of the auditory cortex represents relevant frequency information, but only in AL does this activity directly and causally provide evidence for this decision. However, it is unknown the degree to which ML and AL activity reflects inputs from other brain regions or how it is shaped by local neural processing. To begin to address these questions, we recorded spiking activity, which provides the output signals from a given brain region, and local field potentials (LFPs), which reflect a combination of input, local, and output signals, from ML and
AL, while monkeys performed an auditory frequency-discrimination task. For this task, the monkeys reported whether a sequence of tone bursts contained more high-frequency tone bursts (>1.75 kHz) or low-frequency (<1.75 kHz) tone bursts. The monkeys could report their choice at any time during the presentation of tone bursts. We manipulated task difficulty by systematically changing the proportion of high-frequency and low-frequency tone bursts. The monkeys’ choice accuracy and response times depended systematically on the proportion of high- and low-frequency tone bursts, with faster and more accurate responses for stronger stimuli (i.e., a relatively high proportion of either high-frequency or low-frequency tone bursts). We obtained 79 pairs of spiking activity and LFPs (ML: 35 pairs, AL: 44 pairs) from two monkeys performing the task. Both ML and AL spiking activity was modulated comparably by the frequency content of the tone bursts: spiking activity increased as the proportion of tone bursts with frequencies matching the neuron’s best frequency increased. However, ML LFPs had stronger frequency-dependent modulations than those in AL. Furthermore, a modulation index indicated that ML spiking activity and LFPs had similar frequency selectivity; whereas AL LFPs were less selective than AL spiking activity. Consistent with this observation, the spike-field coherence was stronger in ML than in AL. Together, these results suggest that there is dissociation between spiking activity and LFPs in AL, but not in ML. Based on these findings, we discuss a hypothesis that ML relays sensory inputs to other brain regions, including possibly AL, and AL transforms sensory inputs to a systematically organized representation of sensory evidence used to form the decision.


Poster

508. Auditory Perception, Cognition, and Action

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Topic: D.02. Auditory System

Support: NIH Grant DC009635

NIH Grant DC012557

Charles H. Revson Senior Fellowship in Biomedical Sciences

Title: A synaptic and circuit logic for task engagement in auditory cortex
Authors: *K. KUCHIBHOTLA, J. V. GILL, R. C. FROEMKE; Skirball Inst., NYU Sch. of Med., New York, NY

Abstract: Animals can change their behavior based on behavioral context. A pedestrian will move rapidly away from traffic if she hears a car honk while crossing a street - executing a learned sensorimotor response. The same honk heard by the same pedestrian will not elicit this response if she is seated on a nearby park bench. How do neural circuits enable such flexible interpretation of the same stimuli? Here we show that context-dependent behavioral flexibility controls neural ensembles in the auditory cortex, via cholinergic modulation and local inhibition. Mice were trained to perform a go/no-go operant task in response to pure tones in one context (“engaged”, target tone is rewarded) and listen to the same pure tones but execute no behavioral response in another context (“passive”). When task “engaged”, auditory cortical neurons exhibit broad suppression across a majority of neurons but selective activation of a specific sub-network. Neural responses shifted within a single trial after the context switched. Task performance was reduced by atropine suggesting a role for the nucleus basalis (NB), the primary source of cortical acetylcholine. Using axonal calcium imaging in behaving mice, we show that fibers and terminals projecting from NB to auditory cortex show increased activity in animals only after task learning and during task engagement when compared to passive exposure. Whole-cell voltage clamp recordings in behaving mice showed robust context-dependent changes in inhibitory drive and only modest changes in excitation. Cell-type specific calcium imaging showed that these changes were partially mediated by PV-positive interneurons. Thus, local synaptic inhibition gates long-range neuromodulation from the nucleus basalis to rapidly and reversibly alter auditory cortical output, temporarily decorrelating excitatory and inhibitory inputs, and improving behavioral flexibility. These mechanisms may help explain pathological states, such as post-traumatic stress disorder and addiction, in which flexibility is compromised and innocuous stimuli trigger maladaptive behaviors.

Disclosures: K. Kuchibhotla: None. J.V. Gill: None. R.C. Froemke: None.

Poster

508. Auditory Perception, Cognition, and Action

Location: Hall A

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Topic: D.02. Auditory System

Support: NIH Grant 5R01DC009224
       NIH Grant 5R21DC011659
Title: Contribution of primary auditory cortex to auditory-streaming behavior

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Abstract: A fundamental component of auditory perception is the grouping and segregation of auditory stimuli into distinct perceptual units—a process that is known as “auditory scene analysis”. The auditory brain groups and segregates stimuli based on their spectral, temporal and spatial regularities (or differences). Once grouped, a single perceptual auditory unit is called an “auditory stream”. One important psychophysical task that has illuminated many of the principles and mechanisms underlying a listener’s ability to segregate and group acoustic stimuli is the “streaming” task, in which listeners report whether a sequence of tone bursts at two, alternating frequencies sounds like one “auditory stream” (i.e., “galloping” tones) or two auditory streams. The probability of a listener hearing one or two streams can be manipulated by changing certain parameters of the tone-burst sequences, including (1) the frequency separation between the tones, (2) the listening duration, and (3) the temporal overlap of the tones. Using this tone-burst sequence, we recently demonstrated directly, for the first time, that non-human primates segregate the auditory scene in a manner comparable to human listeners and, consequently, are a valid model of human stream-perception. Here, for the first time, we present findings from extracellular recordings from neurons in the primary auditory cortex (A1) while the animals performed this streaming task. We found that (1) A1 neurons are more broadly tuned during the streaming task than during a passive-listening condition and (2) the firing rate in response to the tone bursts habituates over time. We also find that (3) neural activity is modulated as a function of behavioral report. The degree of choice activity is comparable to that seen in other brain areas that contribute sensory evidence to the decision but do not code the decision itself. Further, unlike previous computational models, we find that choice behavior is not coded by neural habituation but, instead, can be explained by instantaneous firing rate. We discuss these finding in the context of the role of A1 and the hierarchical mechanisms of the ventral auditory pathway.


Poster

508. Auditory Perception, Cognition, and Action

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 508.15/M46
Title: In-ear-EEG indicates neural signatures of effortful auditory processing

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Abstract: Hearing aids (HA) amplify, compress and further process incoming acoustic signals and are fitted to individual hearing impairment. However, HA thus far are unable to completely restore hearing in challenging listening situations, likely because HA operate uncoupled from the listeners' neural and cognitive processes. We here present data in a new approach to gather neural control signals for steering the HA. As a basis we took signals measured in N = 3 participants using Electroencephalography (EEG) recordings from three electrodes placed in the external ear canal (“in-ear-EEG”) alongside with conventional 64 electrodes scalp EEG. The objective of this project was to detect reliable neural signatures of auditory cognitive processing in the in-ear-signals. To this end, we tested two established auditory paradigms: First, a concurrent dichotic oddball paradigm (with 1.4- and 1.8-Hz driving stimulus frequencies) to evaluate the detectability of auditory evoked potentials (AEP) in the in-ear-EEG and second, a cocktail-party, multi-talker scenario to extract neural responses to ongoing speech signals. First, our results show significant P1- and N1-equivalent event related potential (ERP) deflections in the in-ear-EEG on the single subject level in all participants. Second, spectral analysis disclosed evoked components at the two driving frequencies of the oddball paradigm and their higher harmonics. Furthermore, we found a prominent peak in the alpha frequency band for each participant. Third, using cross-correlation techniques, the cocktail-party scenario revealed significant neural phase-locking of in-ear-EEG to the speech envelope. Lastly, validating our approach, there was a significant and spatially distributed correlation of time-domain in-ear-EEG with simultaneously acquired conventional scalp EEG. In sum, by using established challenging-listening paradigms and data analyses, our results prove the feasibility of in-ear-EEG and the availability of neural signatures related to auditory processing. These findings thus provide a promising basis for further research using more real-life listening paradigms and brain-computer-interface (BCI)-geared analyses.

**Poster**

**508. Auditory Perception, Cognition, and Action**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 508.16/M47

**Topic:** D.02. Auditory System

**Support:** DFG Cluster of Excellence 'Hearing4All'

**Title:** The role of parvalbumin-positive interneurons in auditory gap detection

**Authors:** *L. OSTERHAGEN, K. J. HILDEBRANDT;*  
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**Abstract:** Age-related hearing deficits often include impairment of the processing of temporal fine structure, which may be due to deterioration of cortical processing. Age-related structural and functional changes of the auditory cortex have been found within the inhibitory interneuron (IIN) system: The levels of IIN-targeting neurotransmitters are decreased in older mammals. Besides, a decrease of parvalbumin-positive (PV+) IINs, one main group of IINs, had been shown. PV+ IIN in the auditory cortex have been proposed to play an important role for temporal sound processing. We hypothesize that impairments of processing temporal fine structure, which affect primarily older people/mammals, are related to decreased activity in PV+ IIN and that it is possible to enhance the detection of short gaps in noise by artificially activating those neurons. In order to test for the effect of altered PV+ activity, we choose to use optogenetic activation of PV+ cells and test for both physiological and perceptual consequences. We circumvent the interactions of timing of light and sensory stimulus by using stable step-function opsin (SSFO), which can be rendered continuously active and inactive with short pulses of light. By using SSFO, we were able to examine the effects of prolonged low-level and probably more physiologically accurate activation on both neural and behavioral gap detection thresholds. In a first step, we performed electrophysiological recordings in awake, passively listening mice with and without optical activation of PV+ cells. Neural gap detection thresholds for gaps in noise were significantly enhanced both in local field potentials and spiking responses. Units that displayed a significant change in gap detection thresholds also showed increased offset responses at the end of noise stimuli, possibly suggesting a role of cortical offset responses in gap detection. In a second step, we developed an acoustic gap detection task that is embedded in a Go/No-Go paradigm in order to test for perceptual consequences of PV+ manipulation. During the task, continuous noise is presented to the animal. When, a gap of varying length is inserted into the noise stream the mouse has to leave a platform in order to gain a reward. Mice reached
performance level > 80% within 20 training sessions. Behavioral gap detection matched neural
detection thresholds (~ 3 ms). Currently, we are testing behavioral performance with, the activity
of PV+ cells either manipulated through optical stimulation or left untouched. We expect
detection performance to be increased in trials in which PV+ IIINs are artificially activated by
light stimulation as compared to trials without stimulation.

Disclosures:  L. Osterhagen: None. K.J. Hildebrandt: None.

Poster

508. Auditory Perception, Cognition, and Action

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

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Topic: D.02. Auditory System

Support: European research Concil (ERC) ‘Neuroconsc’

INSERM Avenir Program

Fondation Bettencourt Schueller

Title: How do anesthetics affect fMRI responses to auditory stimuli

Authors: *L. UHRIG1,2,3, M. DUPONT4, B. JARRAYA1,2,4;
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of Neurosurgery, Foch Hospital, UVSQ, Univ. Paris-Saclay, Suresnes, France

Abstract: Functional magnetic resonance imaging (fMRI) can be performed either in awake
or anesthetized monkeys. Conducting fMRI under anesthesia is a powerful mean of studying brain
dynamics during consciousness manipulation [1]. However, for a rigorous interpretation of fMRI
data, the anesthesia protocol should ideally use a single hypnotic drug, reproduce the same level
of targeted anesthesia depth, maintain and carefully monitor physiological parameters that are
key for the fMRI signal stability. Moreover, anesthetic agents can directly affect fMRI responses
to sensory stimuli. We first established a protocol designed for non human primate studies that
could address these challenges by using either propofol or ketamine as a single anesthetic agent,
measuring electrical brain activity (EEG) during event-related fMRI study, and maintaining
stable body temperature and end-tidal CO2 among other physiological parameters. Then, we
evaluated the fMRI response to low and high frequency sounds in different arousal states (awake
and anesthesia) in 2 Rhesus macaques for each state. In the awake study, monkeys were trained
to fixate. For the anesthesia study, we used two different anesthetic agents that act on different pharmacological receptors: either propofol (GABA-A agonist) with two levels of sedation (light or deep), or ketamine (NMDA antagonist). The level of sedation states was controlled using both an adapted clinical scale and EEG. Monkeys were scanned in a 3T MRI scanner (Siemens Trio) using MION contrast agent. MR compatible headphones were adapted to monkeys (MR Confon, Germany). Data were analyzed to determine the fMRI activations linked to high and low frequency sounds (SPM5, Matlab). We found reproducible activations of the macaque auditory cortex in the awake state, during propofol anesthesia and ketamine anesthesia. Activations to sounds within the auditory cortex decreased between the awake state and anesthesia, with a major decrease for low frequency sounds under ketamine anesthesia. The extent of activation also decreased most under ketamine anesthesia. This difference may be related to the fact that propofol and ketamine act differently on the neurovascular coupling, or to the fact that the two anesthetics, while keeping feedforward inputs, affects the feedback processes differently. These differences have to be taken in consideration when performing fMRI under general anesthesia.


Poster

508. Auditory Perception, Cognition, and Action

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 508.18/N1

Topic: D.02. Auditory System

Support: DGAPA IN224414-2

Title: Differences in EEG activity between right and left handed on a sound localization task: an exploratory study

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Abstract: Differences in brain organization and brain processing between right and left handed have been described, nevertheless is continue to be an issue of fact. There is also known that sound processing is different for both hemispheres in relation to sound localization. The aim of
this study was to analyze brain response and brain processing during a sound localization task before and after eye movements. We used twenty stimuli (musical note A, 2s each). Stimuli were presented in a classical random block design, for each group (left handed and right handed). After each run, subjects (n=20 male) respond on a PC keyboard according to where they heard the stimuli (right or left side) and at the same time to gaze on the direction they heard the stimuli (right or left side). For eye movements recording, we placed electrodes according EOG. Results showed significant differences on brain activity between left and right handed according to sound localization. There is an increased on absolute power over widespread brain regions for both hemispheres especially on high frequencies, alpha2, beta1, beta2 and gamma higher on right handed. Left handed presented a decrease on absolute power on c4, t4, t5, t6, p4, o1, fz, pz involving high frequencies alpha2, beta1, beta2 and gamma. Inter correlation showed differences between beta1 and gamma higher for left handed. Behavioral data analysis showed higher response on right side comparing pc keyboard answers for both right and left handed, nevertheless there is a major response to left side for eye movements especially for left handed. By the time we might say that brain processing during a sound localization task, is similar for right and left handed according to EEG results, so brain widespread response especially in high frequencies were corroborated. Moreover on behavioral analysis, it seems that on PC keyboard answers and eye movements response, there is a difference denoting that for eye movements, laterality plays an important role that might be related to orientation reflex, so eye movement response is faster and spontaneous in relation to PC keyboard answers where subjects need to be more attend to the task to give an answer. This project is partially financed by DGAPA project IN224414-2. María del Carmen Castro-González is granted by DGAPA project IN224414-2.

Disclosures: M. Castro González: None. Y. del Río-Portilla: None.

Poster

508. Auditory Perception, Cognition, and Action

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 508.19/N2

Topic: D.02. Auditory System

Title: Misophonia: reflecting on self-generated trigger sounds

Authors: *M. EDELSTEIN*, D. BRANG*, B. MONK*, R. ROUW*, V. S. RAMACHANDRAN; 1UC San Diego, La Jolla, CA; 2Interdepartmental Neurosci. Program, Northwestern Univ., Evanston, IL; 3Dept. of Brain and Cognition, Univ. of Amsterdam, Amsterdam, Netherlands
Abstract: Misophonia, literally translated as the “hatred of sound,” is a newly characterized condition where afflicted individuals (misophonics) experience autonomic arousal and intense negative emotions to specific sounds. These “triggers” are typically repetitive sounds generated by another person, including chewing, breathing, tapping, lip smacking and pen clicking. The extreme aversion misophonics experience when exposed to these sounds is characterized by an intense fight-or-flight response which can include emotions of rage, anger, anxiety and panic, far beyond what is typically displayed by non-misophonic individuals. The reactions to these sounds can be so debilitating that many misophonics feel the need to habitually avoid commonplace situations or particular people (including family members), all which can significantly diminish quality of life. As only specific sounds evoke these negative responses, misophonia is distinguished from a general aversion or oversensitivity to sounds (such as hyperacusis). Additionally, misophonia severity appears to be modulated by contextual factors such as sound source, suggesting that there is a strong ‘top-down’ cognitive component to the condition. Anecdotally, many misophonics have reported that they are not bothered when they produce their own trigger sounds with some even employing mimicry as a coping strategy; further, trigger sounds produced by infants or animals were reported to be less aversive than sounds produced by adult humans. In order to empirically validate these anecdotal reports, we performed several assessments that explored whether the misophonic fight-or-flight response is modulated by context. Here we present preliminary behavioral (aversiveness ratings) and physiological testing (galvanic skin response) data gathered from these experiments. This preliminary data reveals that misophonics quickly habituate to self-generated sounds when watching themselves perform these actions in a mirror. Currently there is no standard treatment for misophonia; however, these findings provide important insight towards the development of therapies for attenuating the symptoms associated with the misophonic condition.


Poster

508. Auditory Perception, Cognition, and Action

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

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Topic: D.02. Auditory System

Support: NIH Grant R01 DC005779

Title: Primacy of frequency over amplitude modulation rate in retrieval of auditory memory
Abstract: In order to explore the representation of sound features in auditory memory and the role of dorsolateral frontal cortex (dlFC) in auditory long-term memory, we developed an auditory task that requires information retrieval from an auditory long-term memory (LTM) store. Two ferrets were each trained on two versions of a 3-zone classification task based upon the acoustic features of either: 1) tone frequency (TN-task), or 2) amplitude modulation rate with white noise carrier (AM-task). While tone frequency and AM rate form a continuous distribution, in the LTM tasks both TN and AM sounds were divided into three distinct zones (Low, Middle and High) with clear boundaries. The animals were trained with a positive reinforcement Go/No-Go paradigm, with one stimulus presented in each trial. They learned to approach a waterspout for reward (Go-response) when a sound from the Middle zone was presented, and to avoid a time-out by not licking the waterspout (No-Go response) after a sound from either the Low or High zone was presented. Animals were initially trained on the TN-task, and after reaching behavioral criterion, were then trained on the AM-task. After learning both tasks to criterion, acoustic probes (unrewarded) were presented during task performances. Four sets of probe testing were conducted, in which animals were engaged in: A) the TN-task with AM noise as probe sound, B) the AM-task with the TN as probe sound, C) the TN-task or D) the AM-task with a hybrid combination of AM and TN as probe sounds (i.e. AM modulated tones). The ferrets showed no influence of task-set on probe trial performance i.e. displayed similar discrimination behavioral response to sounds whether they were presented in a probe-free task, or if the same sounds were played as probes while the animals engaged in different task (testing A,B). Our results show that animals were able to simultaneously activate both classification systems during a session with mixed stimuli. However, when the AM-TN combination (hybrid) probes were used, we found that the frequency feature dominated behavioral choice - no matter whether the animals were engaged in either the TN-task or AM-task (testing C,D). These results suggest that the frequency feature has primacy over the AM feature during retrieval of the auditory LTM. We recorded from single units in the dlFC in the different task and test conditions in order to gain insight into the neural basis of these behavioral results and to explore how the frontal cortex encodes, represents, classifies and retrieves the associative meaning of different sensory stimuli during passive stimulus presentation and during performance of multiple tasks.

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Title: Neural substrate for sound symbolism: Visual size judgment with combinations of voiced and voiceless plosives with a vowel “o” or “i”

Authors: *S. ITAGAKI*¹, S. MURAI², K. I. KOBAYASI², J. AURACHER³, H. RQUIMAROUX²;
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Abstract: The sound symbolism is argued to help language acquisition and is possibly related to language evolution. In most of the previous psychology and linguistics researches, stimuli were presented visually with alphabets, and subjects directly answered the impression of the sound.

This study had two goals, 1) verifying whether sound symbolism could be observed even when the sound stimulus was presented aurally, 2) establishing a behavioral paradigm applicable to functional magnetic resonance imaging (fMRI) research. In this experiment, we focused on sound symbolism in visual size. Subjects were all right-handed Japanese native speakers, and they were required to answer visual size difference between standard and target stimulus. Visual stimuli were a gray circle on black background LCD screen, and had 1 type of standard and 6 types of target. Target size was either smaller or bigger than the standard by ±5%, ±10%, or ±20%. Sound stimuli were /bobo/ and /pipi/, and were assumed to have impression of “bigger” and “smaller” respectively, according to previous researches. Congruent trials were defined as those where circle sizes were correlated with the impression of accompanying sounds, and incongruent trials were with opposite stimulus contingency. As a result, reaction times in incongruent condition were longer than congruent condition. Moreover, reaction time differences (= congruent versus incongruent) in 5 % size difference condition is smaller than 10% and 20% size difference condition. These results suggest that sound symbolism does occur even when the sound stimulus was presented aurally. We are currently investigating neural substrate of the sound symbolism in MRI using the same behavior paradigm. The reaction times in incongruent condition under MRI scan were longer than in congruent condition; and the difference of reaction time was longer as visual size difference got greater. In addition, analysis of MR signal showed a steady activation of auditory cortex during the presentation of standard stimulus. These data demonstrate that our paradigm is able to use for fMRI study. However, the blood flow in brain did not appear to differ between congruent condition and incongruent condition across subjects; MRI imaging results shows that the location and amount of activity is different by subjects, suggesting the neural substrate of the sound symbolism could vary between individuals. We will also discuss relationships between the brain differences and individual behavioral differences.

Poster

508. Auditory Perception, Cognition, and Action

Location: Hall A

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Topic: D.02. Auditory System

Support: Wellcome Trust Grant; RES/0164/7500

Title: A brain system for auditory working memory

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Abstract: We examined the human brain system for auditory working memory: the process of holding sounds in mind over seconds. Neural activity was measured using fMRI data acquired at 3T. 16 human subjects listened to a pair of tones followed by a visual cue instructing them to remember one tone after which they were required to maintain that tone for 16s before they were required to compare the remembered tone with a test tone. During the maintenance period, when no sound is present, sustained activation in individual voxels was observed in belt homologues in auditory cortex (planum temporale and lateral part of Heschl’s Gyrus, HG). Sustained activity was also observed in hippocampus and frontal regions including inferior frontal gyrus (IFG). Multivoxel pattern analysis (MVPA) showed that although activity in most voxels in HG was subthreshold, patterns of activity in it could distinguish which of the two tones subjects were maintaining in memory. Above chance classification of maintained tones from patterns of neural activity was also observed in the left IFG. Functional connectivity analysis using psychophysiological interactions (PPI) demonstrated long-range connectivity between posterior auditory cortex and both hippocampus and inferior frontal gyrus during the maintenance period. Our data suggest a system for auditory working memory in which specific representations in the auditory cortex are kept active during the maintenance period by projections from the higher order areas in hippocampus and inferior frontal gyrus. This role of hippocampus in working
memory is in accord with the emerging idea that its mnemonic role is not confined to episodic memory.

Disclosures:  

Poster  
508. Auditory Perception, Cognition, and Action

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Title: White matter microstructure correlates with sensorimotor synchronization

Authors: *T. BLECHER*, I. TAL*, M. BEN-SHACHAR*;  
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Abstract: Sensorimotor synchronization to an external rhythm is a developed ability in humans, particularly evident in dancing and singing. Audio-motor synchronization likely relies on direct or indirect connections relaying information between the auditory and motor brain systems, but the exact pathways that mediate this ability have not been identified to date. The goal of this study is to examine the contribution of fronto-temporal and callosal connections to specific measures of sensorimotor synchronization. Twenty neurotypical adults were recruited for this study (age range 19-40, 9 males). Each participant underwent behavioral and MRI measurements at 3T. Rhythmic synchronization was evaluated via a finger tapping task, in which the participants were asked to tap along with an auditory metrically structured rhythm using their right hand. The meter of the auditory stimulus changed several times during each session and participants had to adjust their tapping pattern to it. For each participant, we quantified the negative asynchrony between the motor taps and the auditory stimuli, as well as the time it took to resynchronize with an altered meter. Using diffusion MRI and deterministic tractography we identified the bilateral arcuate fasciculus, the temporal and the frontal segments of the corpus callosum (CC). We found significant correlations between negative asynchrony and fractional anisotropy (FA) in the temporal-CC and in the left (but not right) arcuate fasciculus. In addition,
FA in the motor-CC correlated with the mean time to resynchronize with an altered meter. To our knowledge this is the first demonstration that diffusivity parameters of white matter tracts are selectively associated with specific audio-motor synchronization measures. We propose that left fronto-temporal fibers are involved in ongoing comparison between motor programs and auditory input, in order to optimize synchronicity between them. Resynchronizing with a new meter engages frontal callosal connections, perhaps involved in inhibiting the automatic tapping to the old meter and switching to a new perceived meter. Our findings offer a possible explanation for recent reports of impaired sensorimotor synchronization in children with developmental speech and language impairments. This association may stem from the reliance of sensorimotor synchronization on the same pathways involved in language skills such as phonological awareness.

**Disclosures:** T. Blecher: None. I. Tal: None. M. Ben-Shachar: None.

**Poster**

508. Auditory Perception, Cognition, and Action

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program/#Poster#:** 508.24/N7

**Topic:** D.02. Auditory System

**Support:** The Ministry of Trade, Industry and Energy (Grant number: 10041629)

KIST Institutional Program (Grant number: 2E25710)

**Title:** Object-based sound signal classification

**Authors:** *Y. LIM, J. CHOI;
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**Abstract:** Most sound recognition method often begins with the acoustic feature vectors that are obtained from the spectral amplitude of sound signal. Although several studies have shown the importance of the phase in speech coding and its benefit for improving the performance of the recognition system [1]-[4], spectral phase information has been neglected. Recently, we have proposed an object-based time-frequency representation in which the elementary units are adaptive contours that define the phase coherent areas in time-frequency plane [5]-[7]. From simple static signals to natural sounds such as birdsong, we have shown that this method can adaptively represent signals, such that individual components of a sound stream are represented in their own natural time-scales, or bandwidths. Here, we introduce a new method for sound
signal classification with the object-based acoustic feature vector that does not take account of
spectral amplitude of signal. In the method, statistically significant contour objects from multiple
analysis parameters are used to build an object-based acoustic feature vector, which represents
the temporal transition of analysis parameters for the selected objects. We have applied the
proposed method to represent the various kinds of sound in our natural environment such as
human scream, dog, and so on. Since there is no amplitude information involved in the
recognition process, we believe that this new forms of object-based acoustic feature vector could
allow for the construction of an amplitude invariant recognition system. Finally, we speculate that
neural auditory perception may involve a similar feature selection process by utilizing a parallel
and redundant computations along the auditory pathway. [1] A. C. Lindgren, M. T. Johnson, and

Disclosures: Y. Lim: None. J. Choi: None.

Poster

508. Auditory Perception, Cognition, and Action

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 508.25/N8

Topic: D.02. Auditory System

Support: BGN – Berufsgenossenschaft Nahrungsmittel und Gastgewerbe

Title: An acoustic discrimination task can be used to monitor psychical stress and enhanced
cognitive demands in hearing impaired people

Authors: C. GORF1, R. HUONKER1, E. EMMERICH2, *(F. RICHTER2);
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Abstract: Hearing impaired people complain of psychical stress that is enhanced by listening or
by solving auditory recognition tasks. An increased level of psychical stress is assumed to
contribute to the extra-aural effects of noise, i.e. increased heart rate, hypertension or disturbances in the endocrine system. Here we wanted to establish a paradigm to test whether psycho-vegetative parameters and central processing of auditory signals are changed in hearing impaired people and whether these changes are aggravated if auditory signals are masked by noise. In two groups of young adults (one group normal hearing, 20 participants; one group with moderate hearing impairments above 20 dB SPL in the frequency range of 3-4 kHz, 16 participants) we recorded the magnetoencephalogram (MEG) and auditory evoked magnetic fields in order to analyze the distribution of the sources and to perform a wavelet analysis. We recorded the classical EEG, the electrocardiogram, arterial oxygen saturation and breathing frequency. As a marker for psychical stress we measured the concentration of cortisol in the saliva prior to and after the experiments. Auditory stimuli were tone pips with slightly modified frequencies presented at an intensity of 65 dB SPL. In a “one back task” the subjects had to decide, whether the next to last pip had a higher or lower frequency than the last pip, and correct responses were rewarded. In a second series the tone pips were overlaid by random noise and the same task was performed. In the hearing impaired group the detection level for higher versus lower tone pips was significantly worse, but error rates and reaction times were lower than in normal hearing people, thus indicating a higher alert level. The rates of missed answers were higher, and mean EEG-frequency at rest shifted to higher frequencies in the alpha range in the hearing impaired group. In this group the heart frequency was higher even at rest but did not further increase during the auditory task. In hearing impaired participants the content of cortisol in the saliva at rest was higher than in the control group and remained at still higher levels during the auditory task. We conclude that hearing impairment per se induces psychical stress at rest and in workaday life, and this stress enhances the cognitive demand during auditory tasks in profession and leisure activity. This higher mental load has to be considered in employment medical care particularly with regard to hearing impairment.

Disclosures: C. Gorf: None. R. Huonker: None. E. Emmerich: None. F. Richter: None.

Poster

508. Auditory Perception, Cognition, and Action

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 508.26/N9

Topic: D.02. Auditory System

Support: IR01 DC013961

Title: Combined effects of frequency and location differences on auditory streaming
Authors: J. LEE¹, S. BENNUR¹, Y. I. FISHMAN², *Y. E. COHEN³; ¹Univ. of Pennsylvania, Philadelphia, PA; ²Albert Einstein Col. of Med., Bronx, NY; ³Dept. Otorhinolaryngology-Head Neck Surgery, Univ. of Pennsylvania Sch. of Med., Philadelphia, PA

Abstract: A fundamental aspect of hearing is the perceptual organization of time-varying acoustic inputs to form auditory objects corresponding to sound sources in the environment (i.e., “auditory scene analysis”). One process contributing to auditory scene analysis involves the integration or segregation of sequential sound inputs based on acoustic cues such as frequency; this is a phenomenon known as auditory streaming. Another process utilizes spatial cues to infer auditory objects based on their location. Whereas many studies have separately examined auditory streaming and sound localization, few studies have tested how these two processes interact in auditory scene analysis. Here, we examined this issue by conducting a series of psychophysics experiments in human subjects aimed at identifying the relationship between auditory-streaming cues, sound localization, and auditory scene analysis. Specifically, we presented sequences of alternating tone bursts that varied in frequency, location, and sound level. Subjects were asked to report when they heard a deviant louder tone burst. We varied task difficulty by changing either the frequency difference between the tones, the spatial separation between the tones, or both. To our knowledge, this is the first human psychophysical study to examine the interactive effects on auditory streaming of three fundamental acoustic cues used in auditory scene analysis: differences in frequency, azimuth, and intensity.

Disclosures: J. Lee: None. S. Bennur: None. Y.I. Fishman: None. Y.E. Cohen: None.

Poster

508. Auditory Perception, Cognition, and Action

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 508.27/N10

Topic: D.02. Auditory System

Support: DFG Cluster of Excellence EXC 1077/1 "Hearing4all"

Title: Temporal dynamics of processing task-relevant and irrelevant sound feature changes

Authors: S. PUSCHMANN¹, R. J. HUSTER², *C. M. THIEL¹; ¹Univ. of Oldenburg, Oldenburg, Germany; ²Univ. of Oslo, Oslo, Norway

Abstract: Previous fMRI studies demonstrated change-related activations in human auditory cortex but also in frontal and parietal brain regions (Sabri et al., 2011). Due to its low temporal
resolution, fMRI can, however, not capture the temporal dynamics of the activation spread across the different brain regions. The interplay of auditory sensory regions with fronto-parietal brain regions during deviance processing has therefore not been investigated in detail. We aimed to solve this issue by concurrently measuring fMRI (3T, TR=1.5 s) and EEG (64-channel). We used an auditory stimulus categorization task, in which participants (N=24, right-handed, normal-hearing) had to sort successively presented complex tones (mean SOA = 1,500 ms) via button press into one of two stimulus classes, either depending on the fundamental frequency (F0 = 500/630 Hz) or the stimulus duration (150/300 ms). The categorization rule was randomly switched after variable delays of 12 to 48 seconds as indexed by a visual cue. Functional MRI responses were analyzed for task-relevant and irrelevant frequency changes. Change-related activity was found in secondary auditory cortex, but also in the superior parietal lobe, the temporo-parietal junction, the inferior and medial frontal gyrus, the frontal eye fields, and the insula. BOLD responses in the superior parietal lobe, the inferior frontal gyrus, and the insula were increased for irrelevant as compared to task-relevant changes, whereas no differences were observed in any of the other brain regions showing change-related responses. EEG source reconstruction (sLORETA) was used to extract the time course of neural activity from all brain regions showing change-related activity in fMRI. An analysis of source activation peak latencies revealed that activation spreads from auditory sensory areas and parietal brain regions to the insula and subsequently to inferior and medial frontal gyrus, before re-activating parietal regions and auditory cortex. Supporting the fMRI results, differing response amplitudes between behaviorally relevant and irrelevant changes were evident in the insula and, later, in the superior parietal lobe. Our data show a propagation of change-related activity from auditory sensory areas into parietal and then frontal brain areas of the dorsal and ventral attention network. This finding complements previous work on auditory attention switching showing similar spatio-temporal dynamics (Green et al., 2011). First relevance-related differences emerged at the level of the insula, supporting the view that this structure is involved in evaluating the behavioral relevance of sensory input (Menon & Uddin, 2010).

Disclosures: S. Puschmann: None. R.J. Huster: None. C.M. Thiel: None.
Title: Hierarchical neuronal representations of sound categories in human brain

Authors: Y. ZHANG1, Y. DING1, *J. HUANG1, W. ZHOU2, Z. LIN6, B. HONG1, X. WANG3,5;  

Abstract: The discrimination and recognition of different categories of sounds are assumed to be processed by functionally specialized cortical areas. However, the specific locations of these areas and the neural mechanisms underlying sound discrimination and recognition are largely unknown. We used both electrocorticographic (ECoG) and functional magnetic resonance imaging (fMRI) techniques for brain activity recording. Five different categories of voice and non-voice complex sounds (including voiced speech, scrambled voiced speech, non-speech voice sounds, animal vocalizations and natural sounds) were used to investigate the roles of various cortical areas in processing sound categories. Our results revealed potential hierarchical organizations of sound category processing. Neural signals recorded from individual ECoG electrodes in five epilepsy patients showed different selectivity to distinct sound categories. After mapping the ECoG signals (60-140Hz) onto the average brain surface, we identified four responsive sites in the left hemisphere (anterior STG, middle STG, posterior STG and middle pre-central areas) and three responsive sites in the right hemisphere (anterior STG, middle STG, posterior STG) that exhibited sound category selectivity. Furthermore, using peak latency and Granger causal connectivity analysis (GCCA), we observed a progression of cortical activity corresponding to sound category from middle STG to anterior STG and from middle STG to posterior STG, respectively. More specifically, we found anterior STG of the left hemisphere only responded to Chinese voiced speech, which may indicate its involvement in the processing of lexical information. We also found the activation of middle pre-central areas in the left hemisphere, which may indicate the implicit transformation of acoustic representation to sensorimotor representation. These findings suggest a potential hierarchical processing of sound category in the human brain.

Disclosures: Y. Zhang: None. Y. Ding: None. J. Huang: None. W. Zhou: None. Z. Lin: None. B. Hong: None. X. Wang: None.

Poster

508. Auditory Perception, Cognition, and Action

Location: Hall A
Title: Perceptual restoration of missing speech sounds in human auditory cortex

Authors: *M. K. LEONARD¹, M. J. SJERPS², M. O. BAUD², E. F. CHANG¹;
¹Neurolog. Surgery, ²Neurol., UCSF, San Francisco, CA; ³Linguistics, UC Berkeley, Berkeley, CA

Abstract: Humans rarely hear speech under ideal listening conditions. Yet even in noisy environments, listeners can understand spoken input, and may even be unaware that segments of the signal are not physically audible. The phoneme restoration effect is a robust phenomenon in which the acoustic signal for a phoneme in a word or sentence is removed from the speech stream, and replaced by a non-speech signal like broadband noise. Rather than perceiving a gap in the speech signal, listeners report hearing the missing phoneme, and are typically unable to report which sound was replaced. This phenomenon is central to the debate over whether speech perception networks utilize feedback mechanisms to alter lower-level representations based on higher-order knowledge in real time. We recorded neural activity directly from the surface of the brain in human subjects implanted with multi-electrode arrays as part of their treatment for medically refractory epilepsy. Participants listened to words where one phoneme was replaced with broadband noise, and the original versions of those words. The missing phoneme could restore to only two sounds to create real English words (/fæ#tr/ restores to “factor” or “faster”), and listeners reported which word they heard on each trial. Despite hearing the same physical stimulus (/fæ#tr/), subjects reported different percepts on individual trials. Superior temporal gyrus electrodes showed responses to noise trials that matched listeners’ subjective perception. For example, when subjects reported hearing the word “factor”, the neural response to the noise closely matched the neural response to the plosive /k/ when subjects heard the original stimulus, /fæktr/. Across lateral temporal and frontal lobe electrodes, perception-matched responses to
noise stimuli occurred during the same time window that showed differential responses to the original phonemes (/k/ vs. /s/). Stimulus spectrogram reconstruction revealed that neural populations represented the spectrotemporal features of the perceived phoneme, indicating online warping of the acoustic representation. Finally, pre-stimulus fronto-temporal neural activity predicted subsequent perception, suggesting both bottom-up and top-down causal influences on subjective experience. Resilience to acoustic occlusion is a fundamental component of speech perception in natural conditions. These results demonstrate that missing acoustic content is not simply inferred from contextual cues, but is created in auditory cortex during ambiguous input. This provides direct evidence for interactive models of speech perception, in which lower-level representations are altered online.


Poster

508. Auditory Perception, Cognition, and Action

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 508.30/N13

Topic: D.02. Auditory System

Support: Natural Sciences and Engineering Research Council of Canada Grant RGPIN-2014-04465

Title: Sensorimotor representations in the language network during sentence repetition

Authors: *K. MÜSCH¹, T. A. VALIANTE², K. HIMBERGER¹, C. J. HONEY¹;
¹Dept. of Psychology, Univ. of Toronto, Toronto, ON, Canada; ²Div. of Fundamental Neurobio., Toronto Western Res. Inst., Toronto, ON, Canada

Abstract: Motivation: Sensorimotor response patterns are observed in both left and right hemispheres during basic linguistic processes, such as phoneme perception and production (Cogan et al. 2014). Why are joint sensory and motor responses observed during perceptual and productive aspects of language? One fundamental aspect of real-life language is its temporally extended structure, and higher-order sensory and motor systems may support language comprehension by organizing and integrating sequential patterns of information over many seconds of time. To investigate the role these higher-order systems in the process of temporal integration, it is important to map the spatial distribution and functional signatures of sensory and motor response patterns during temporally extended, meaningful language perception and
production. Methods: We recorded electrocorticographic (ECoG) signals from the lateral surface of the human cerebral cortex, in seven patients with pharmacoresistant epilepsy. Participants performed a sentence repetition task: on each trial (i) two sentences were presented sequentially, (ii) participants silently rehearsed the second sentence, and (iii) finally they repeated the second sentence aloud. To estimate activity of neuronal populations beneath each electrode during task performance, we measured fluctuations of broadband power (70-200 Hz) in the voltage trace.

Results: During the initial perception of the sentence, we observed bilateral responses both in sensory regions (the superior temporal gyrus) as well as the motor system (dorsal motor and premotor cortices). During the silent rehearsal phase, we observed that (i) the dorsal motor and premotor activations were augmented by recruitment of ventral motor sites; and (ii) the activity in the superior temporal gyrus was shifted posteriorly towards lexico-semantic regions. Finally, during repetition, activity was focused in bilateral dorsal motor and premotor cortex.

Conclusions: These results suggest that the representation of temporally extended information in natural linguistic contexts is supported by higher-order sensorimotor circuits within dorsal and ventral precentral sites as well as by circuits in the posterior temporal cortex. Our findings complement prior observations of sensorimotor transformations supporting phoneme production and perception, and extend them to the context of temporally extended linguistic perception and production. References: Cogan et al. (2014). Sensory-motor transformations for speech occur bilaterally. Nature 507, 94-98.


Poster

509. Cross-Modal Processing: Temporal Factors

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 509.01/N14

Topic: D.03. Multisensory Systems

Support: Natural Science Foundation of China (11174316, 31200760)

National High Technology Research and Development Program of China (863 Program) (2012AA011602)

Title: Temporal ensemble coding in sub-second sensory events: simultaneous EEG and fNIRS signatures
Authors: *L. CHEN*1,2, L. GUO3, H. YILTIZ1, M. BAO1;
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Abstract: For decades, investigations into the encoding of temporal precision have mainly focused on the paradigm of direct perceptual estimation with two paired sensory events. Modality temporal precision hypothesis states precisions of temporal processing differ across visual and auditory modalities, with the former higher and latter lower. The present study examined the ensemble coding towards a sequence of sensory events containing multiple sub-second inter-intervals. We implemented two experiments. In Experiment 1, after hearing a sequence of sound beeps (5 sounds, with mean inter-interval of 600 ms), participants made perceptual estimation with respect to whether the time interval between the last beep of the sequence and the target stimulus (beep or visual flash after the sequence) was shorter or longer than half, sharp or double 'mean' interval of the preceding inter-intervals of sound beeps. In Experiment 2, immediately after the preceding sound sequence, participants produced half, sharp or double 'mean' interval instead. The results have shown general over-estimation of the mean interval for multiple sensory events. Importantly, in both experiments, variances of estimated interval (hence the 'encoding') for the sharp mean intervals were larger than those in 'half' and 'double' conditions, although our brain embraces remarkable ability of temporal ensemble coding. Corresponding to the behavioral tasks, we recorded simultaneously EEG signals and brain activities by functional Near-Infrared-Spectroscopy (fNIRS). In prefrontal areas, when the target stimulus was auditory beep, the estimation of target interval elicited more negative going ERPs than the visual flash target did, indicating the encoding of auditory interval was more precise and but more task-demanding for processing. Moreover, the sharp-cycle sampling led to more negative contingent negative variation (CNV) components than the half-cycle and double-cycle samplings did, and the changes of concentration of deoxyhemoglobin (HbR) was largest in sharp-cycle sampling condition. Taken together, the results indicate that characteristics in temporal ensemble coding of sub-second sensory events could be captured by EEG and fNIRS signatures.

Disclosures: L. Chen: A. Employment/Salary (full or part-time); Department of Psychology, Peking University. L. Guo: None. H. Yiltiz: None. M. Bao: None.

Poster

509. Cross-Modal Processing: Temporal Factors

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 509.02/N15
Title: Perceived timing of multisensory events during a fall

Authors: *M. Barnett-Cowan, K. Duggan, J. Lupo;
Dept. of Kinesiology, Univ. of Waterloo, Waterloo, ON, Canada

Abstract: About one-third of seniors fall each year, with hip fractures being the most common fall injury and about 20% of injury-related deaths among seniors can be traced back to a fall. The current clinical paradigm is to assess unisensory function (e.g., visual acuity), and correct unisensory impairment (e.g., corrective lenses), with the intent of preventing falls. However, it may be that the clustering of visual impairment with other sensory impairments and/or the way the central nervous system (CNS) integrates multisensory cues differently during aging is what puts adults in later life at a higher risk. The CNS receives information about the environment from all the senses. To safely interact with the environment, the CNS must quickly make sense of this converging multisensory information in order to form a reliable and accurate percept with which to guide decision-making and behaviour. As we age, however, natural changes occurring in the CNS affect the way the senses provide accurate and reliable information about the world in timely fashion. While it is well understood that the senses become less sharp as we age, how the CNS continues to integrate multisensory cues with unreliable sensory information available in later life is less clear. Older adults have trouble separating multisensory events in time, an observation that cannot be entirely accounted for by an age-related reduction in unisensory detection thresholds, potentially explaining why many sensory-related challenges experienced in later life (e.g., decision-making, communication, balance control) persist despite the use of corrective devices designed to address unisensory loss. For example, it is relatively unknown how conscious awareness of sensory processing changes during a fall. Common anecdotal reports of falling suggest that people often report distortions in their perception of time with little to no recollection of what occurred during the fall. Our work has previously shown that the perceived onset of self-motion is slow compared to the other external sensory events. Here I will review recent studies focused on the perceiving timing of multisensory events during a fall. The results show perceptual delays of fall onset and distorted duration estimation that further change with age. These results and future work will be discussed in the context of developing new falls prevention assessment techniques to help prevent falls in later life.

Disclosures: M. Barnett-Cowan: None. K. Duggan: None. J. Lupo: None.
Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 509.03/N16

Topic: D.03. Multisensory Systems

Support: NIH Grant T32 MH064913

NIH Grant T32 MH064913

NSERC Banting Postdoctoral Fellowship

Title: Audiovisual integration in cochlear implant users

Authors: *I. BUTERA*1, R. A. STEVENSON2, R. H. GIFFORD2, M. T. WALLACE1;
1Vanderbilt Brain Inst., 2Hearing and Speech Sci., Vanderbilt Univ., Nashville, TN; 3Psychology, Univ. of Toronto, Toronto, ON, Canada

Abstract: Cochlear implants (CIs) are highly successful neuroprosthetic devices that can enable remarkable proficiency in speech comprehension. However, most CI users still struggle to communicate in noisy environments. Audiovisual integration is known to play an important role in boosting speech intelligibility in noise, yet clinical evaluation of a user’s proficiency with a CI is typically restricted to auditory-only measures. The purpose of this study is to assess audiovisual integration in children with CIs and relate this ability to other measures of auditory and visual perception and speech recognition. Participants included 15 CI users and 15 age-matched controls between 6 and 18 years old. Measures included an audiovisual speech-in-noise task, the McGurk Illusion, and psychophysical assessments of audiovisual temporal acuity using either a complex audiovisual stimulus (i.e. the recorded utterance of the syllable “ba”) or a simple audiovisual stimulus (i.e. a flashed circle and 1kHz tone pip). By varying the stimulus onset asynchrony (SOA) between the respective auditory and visual components of these pairings and having subjects perform a simultaneity judgment, we are able to define a subjective “temporal binding window” of audiovisual integration. We hypothesized that temporal acuity and the fusion of auditory and visual speech tokens would differ between CI users and controls and generalize to measures of speech in noise intelligibility. Our data reveal four preliminary findings: (1) significant differences in the SOA at which perceived simultaneity peaked (i.e., the point of subjective simultaneity) for the simple audiovisual stimulus (62 ms for CI v. 22 ms for control; p=0.02), (2) no significant group differences in the width of the temporal binding window with either simple or complex audiovisual stimuli, (3) decreased reports of the fused, illusory token in the McGurk task for CI users (31% v. 75%; p=0.01), and (4) a strong correlation between the report of fused, illusory tokens in the McGurk task with the percentage of correctly identified phonemes in an audiovisual word recognition task presented at a 0dB signal-to-noise ratio (r²=0.64, p<0.01). Taken together, these results suggest a greater visual bias...
in perception at multiple levels of sensory processing in CI users and highlight practical benefits for enhancing audiovisual integration in realistic listening environments.

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**Poster**

**509. Cross-Modal Processing: Temporal Factors**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 509.04/N17

**Topic:** D.03. Multisensory Systems

**Support:** The Scientific and Technological Research Council of Turkey (TUBITAK Grant 113K547)

**Title:** Crossmodal interactions in the timing of a visual event: An EEG study

**Authors:** *H. KAFALIGONUL*\(^1,2\), U. KAYA\(^3\);
\(^1\)Natl. Magnetic Resonance Res. Ctr. (UMRAM), Bilkent Univ., Ankara, Turkey;
\(^2\)Interdisciplinary Neurosci. Program, Bilkent Univ., Ankara, Turkey; \(^3\)Informatics Institute, METU, Ankara, Turkey

**Abstract:** The integration of information from different senses is central to our perception of the world including the fundamental attributes of space and time. Audiovisual interactions have been particularly well studied in this context and various illusions have demonstrated strong influences of audiovisual interactions on our final perception (Chen & Vroomen, 2013). An interesting phenomenon is temporal ventriloquism, in which sounds drive the perceived timing of visual events. Even though temporal ventriloquism has been well studied at the perceptual level, the neural processes underlying auditory influences on visual timing are still unknown. In the current study, we investigated the neural correlates of temporal ventriloquism by collecting behavioral and EEG data in tandem. During the experimental sessions, a brief visual bar (50 ms duration) was presented either on the right or the left of a central fixation point. The visual stimulus was accompanied by an auditory click (20 ms duration) with varying stimulus-onset asynchrony (-160 ms < SOA < 120 ms). The auditory click was introduced either before...
(SOA<0) or after (SOA>0) the visual bar. Observers (n=12) were asked to report the location of the visual bar as soon as it was seen. As expected by temporal ventriloquism, the reaction times required to discriminate the location of visual stimulus were significantly modulated by auditory timing. Reaction times increased linearly as the SOA values were increased. In agreement with recent reports (e.g., McDonald et al., 2013), the auditory click elicited ERPs over early visual cortex (occipital electrodes). Moreover, interactions (mostly super-additive) between auditory and visual responses were observed and the magnitude of these interactions varied with SOA. Time-frequency analysis revealed that the audiovisual interactions led to an increase in 4-12 Hz (theta and alpha bands) power when the SOA value was between -80 and 80 ms. More importantly, for this range of SOA values, we also found that changes in the temporal dynamics of 4-12 Hz power that paralleled behavioral data: the latency was increased linearly as the SOA was increased. Together, our results, in combination with accumulating evidence (Naue et al., 2011; Romei et al., 2012), support an important role for low-frequency oscillations in crossmodal interactions at early stages of perceptual processing.

Disclosures: H. Kafaligonul: None. U. Kaya: None.
from a group of participants, which can conceal substantial between- and within-subject variability in the processing of audiovisual information. Little work has focused on better understanding the brain bases for trial-by-trial differences in audiovisual perception. In the current study, we investigated the neural correlates of trial-by-trial perceptual variation, within the context of a detection task. Participants were asked to detect visual targets (presented alone or in combination with a tone), and to indicate their location (i.e., central vs. peripheral visual field). Response accuracy and reaction times were collected for each trial. Concurrently, high-density EEG recordings were carried out, in order to identify differences in both response strength (i.e., global field power) and neural generator configuration (i.e., topography) of the electrophysiological signals. Analyses focused on identifying differences associated with trials exhibiting multisensory perceptual facilitation compared to identical trials where no perceptual enhancement occurred, as well as detailing how this is affected by target location and stimulus structure (i.e., auditory vs. audiovisual). Preliminary findings suggest that audiovisual interactions within the peripheral visual field are dependent on visual target contrast polarity (i.e., bright vs. dark), leading to differential gains in the efficacy of stimulus processing (quantified as smaller speed-accuracy tradeoffs). EEG data analyses aim at understanding the neural network changes involved in the early, perceptual processing stages of multisensory events that determine the trial-by-trial speed-accuracy tradeoff observed at the behavioral level.


Poster

509. Cross-Modal Processing: Temporal Factors

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 509.06/N19

Topic: D.03. Multisensory Systems

Title: Audiovisual spatiotemporal binding windows in depth

Authors: *J.-P. NOEL, M. WALLACE;
Vanderbilt Univ., Nashville, TN

Abstract: The integration of information across different sensory modalities is known to be dependent on the statistical characteristics of the stimuli that are combined. Namely, the closer in space and in time unisensory (e.g., visual, auditory) stimuli are from one another, the more likely they are to be integrated or bound. Recent psychophysical work has focused on the characterization of audiovisual ‘temporal binding windows’ by asking subjects to judge the simultaneity of paired auditory and visual stimuli presented at different stimuli onset
asynchronies. In this manner, an estimate of the temporal interval over which subjects are highly likely to perceive these as synchronous can be derived. However, the manner in which spatial features drive multisensory binding - and more specifically the characterization of 'multisensory spatial binding windows' analogous to temporal binding windows - is less well understood. Most importantly, an understanding of how multisensory temporal and spatial binding windows interact with one another is missing. Such information is crucial if we are to fully understand how dynamic stimuli in our world are integrated. In the current project we set out to define: 1) temporal, 2) spatial, and 3) spatio-temporal multisensory binding windows in the same subjects by offsetting audio-visual stimuli in either time, space, or both, and asking subjects to judge either their simultaneity, co-localization, or the occurrence of a single event. In the spatial domain, stimulus location was varied in the dimensions of azimuth, elevation, and depth. In the temporal domain, stimuli were separated by asynchronies ranging from 250ms to 0ms. Results reveal that while spatial binding windows enlarge as stimuli are move farther away from subjects, the opposite pattern is exhibited by the temporal windows. These results illustrate that the spatial and temporal filters through which the perceptual world is created are strongly interconnected, and that stimulus depth, whose processing is likely to be strongly modulated by the constructs of peripersonal and extrapersonal space, plays an important role in shaping these filters.


Poster
509. Cross-Modal Processing: Temporal Factors

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 509.07/N20

Topic: D.03. Multisensory Systems

Support: KAKENHI 25-6091

Title: Discrimination of auditory and audio-visual time intervals.an event-related potential study on effects of task difficulty

Authors: *E. HASUO¹, E. GONTIER², T. MITSUDO², Y. NAKAJIMA², S. GRONDIN³;
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Abstract: Discriminating short and long time intervals is easy when the beginning and the end of the intervals are marked by signals of the same sensory modality (intra-modal), but becomes much more difficult when they are marked by signals of different modalities (inter-modal). The
present study examined the neural correlates of such difficulty levels using event-related potentials. Time intervals were marked either by two auditory signals (AA) or by an auditory and a visual signal (AV), and there were two levels of discrimination difficulty (easy and difficult). A negative component (contingent negative variation), which is reported to reflect time perception, appeared at fronto-central sites during the interval presentation. This component was larger for AA than for AV, and was not influenced by discrimination difficulty. A principal component analysis was performed on data from all electrodes sites and all conditions. The first principal component was distributed over a wide area from fronto-polar to central sites with differences between modalities and difficulty levels appearing after 250 ms from the beginning of the time interval. The third principal component was distributed at parieto-occipital sites, and differences between difficulties within the AA condition appeared at around 300-500 ms after the termination of the interval. The second principal component had opposite tendencies between the fronto-polar and the central sites, and showed surprisingly similar time courses in the component scores for all conditions until the end of the time interval. The first and the third principal component seemed to capture differences in brain activity patterns between sensory modalities and difficulty levels, whereas the second principal component seemed to capture a common activity pattern related to the temporal processing independent of the sensory modalities.

Disclosures: E. Hasuo: None. E. Gontier: None. T. Mitsudo: None. Y. Nakajima: None. S. Grondin: None.

Poster

509. Cross-Modal Processing: Temporal Factors

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 509.08/N21

Topic: D.03. Multisensory Systems

Support: ANR SENSEMAKER

Title: Non-symmetric auditory-visual interactions at perceptual and cortical levels in mice

Authors: *T. DENEUX, A. KEMPF, B. BRICE; CNRS UPR 3293, Gif-sur-Yvette, France

Abstract: Although auditory responses have been reported in mouse primary visual cortex (V1), their computational and perceptual role in a multisensory context remains elusive. In particular, it is unclear whether specific multimodal information is channeled through these responses. To address these questions, we recorded neuronal populations in V1 using 2-photon imaging in
awake, GCAMP6-transfected mice, during 2 sec drifting gratings and looming or receding visual and/or auditory stimuli. Among 3,500 recorded V1 neurons, 25% responded to sounds. In contrast we did not find visually-responding neurons in the primary auditory cortex. A large part of auditory responses in V1 were non-specific onset responses and combined linearly with the visual responses, although 7% of the neurons exhibited non-linear bimodal summation properties and appeared to signal specific combinations of bimodal stimuli. Interestingly, auditory population responses correlated strongly with the nonspecific onset visual responses. To assess the perceptual impact of this cross-modal activity, we trained mice to discriminate between looming and receding disks in a head-fixed Lick/No lick protocol, first without sounds. Mice tended to lick on the onset of both visual stimuli, as predicted by the poor specificity of V1 onset responses, and learned to adjust their licking during the more specific phase of V1 responses (about 500 ms after onset). Interestingly, the initial unspecific licking behavior was spontaneously generalized to previously untrained sounds, reflecting the similarity of auditory and visual onset responses in V1, as we demonstrate using a simple computational model of the task. Altogether our results show that specific information about the multimodal context is present in V1, but that the first order impact of auditory signals on mouse visual representation and on perception in a simple task is a boosting of stimulus onset saliency. We also highlight that multisensory integration exhibits complex, non-symmetrical, interactions where one modality can dominate on another.

**Disclosures:** T. Deneux: None. A. Kempf: None. B. Brice: None.

**Poster**

509. Cross-Modal Processing: Temporal Factors

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 509.09/N22

**Topic:** D.03. Multisensory Systems

**Title:** Investigating the influence of visual deprivation on multisensory processing in the superior colliculus

**Authors:** *L. KURELA¹, J. KRUEGER-FISTER¹, M. WALLACE²;
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**Abstract:** Combining information from different sensory modalities is a prerequisite for navigation of the external world. The superior colliculus (SC) is a central hub for multisensory processing. Over half of the neurons in the intermediate and deep layers of the cat SC receive convergent information from two or more sensory modalities, and many of them transform these
different inputs. Developmental studies have shown that multisensory SC neurons mature gradually during early postnatal life. This maturation is critically dependent upon sensory experience, and elimination of visual experience via dark-rearing results in the inability of SC cells to integrate their multisensory input. Restoration of visual experience in adulthood has relatively minimal effects on multisensory processing in these animals, with most neurons still lacking integrative capacity and suggesting the presence of a sensitive period for multisensory experience. If such is the case, then changes in sensory experience only during adulthood should have little effect on multisensory processing. To examine this, cats were reared under normal sensory conditions until adulthood (i.e. for approx. 6 months) and then transferred to a visually deprived environment for an additional 6 months (6+6). Extracellular recordings of local field potentials (LFP) and multi-unit activity (MUA) were conducted using a 24-multichannel U-probe from the SC of these 6+6 animals, and compared with recordings from normally-reared (NR) and completely dark-reared animals (DR). These experiments revealed that although multisensory integration remains in intermediate and deep layer neurons following adult visual deprivation, less of these multisensory neurons are present. When comparing the integrative capacities of the neurons from 6+6 animals with those of NR and DR animals, substantial differences were found, most notably in the response amplitudes and response latencies to unisensory (visual, auditory) and multisensory stimulus presentations at both the LFP and spiking levels. Additionally, current source density (CSD) analyses showed laminar differences in processing between the three groups. Collectively, these findings show that adult (multi)sensory experience remains an important factor in the preservation of normal unisensory and multisensory processing in SC neurons.


Poster

509. Cross-Modal Processing: Temporal Factors

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 509.10/N23

Topic: D.03. Multisensory Systems

Support: ERC

Title: Neural and behavioural signatures of the temporal integration window for auditory and facial-tactile stimulation

Authors: *J. M. ZUMER, T. P. WHITE, U. NOPPENENY;
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**Abstract:** Synchrony between sensory signals is a fundamental cue indicating whether they were caused by a common source. Previous studies indicated that integration may occur directly between auditory and somatosensory cortices via oscillatory phase resetting, rather than through higher integration regions. Tactile stimulation to the face is particularly alarming and requires a fast decision if harmful. We sought to determine the effect of temporal synchrony between auditory and tactile stimuli to the face on behavioural measures and neural signatures measured by EEG. We presented audio-tactile stimuli (both 200ms duration) to young, healthy adults (N=25; 3 excluded). The tactile signal was the tip of a fibre optic cable, driven by pressurised air, presented to the left cheek. Sounds were delivered via foam in-ear inserts with plastic tubing; the auditory target was given a left bias through inter-aural amplitude (IA) cues and presented on top of constant, balanced-IA background (MRI) noise. Ten trial types were presented randomised: tactile alone, auditory alone, null stimulation, and 7 multisensory asynchronies (+/-500ms, +/-70ms, +/-20ms, 0ms). On day 1, participants were asked to respond as quickly as possible when they sensed stimulation in either modality. On day 2, 64-channel EEG data was recorded as participants passively received the same stimulation. The redundant target effect was found in reaction times (RT): participants responded faster to (near) synchronous multisensory compared to unisensory or +/-500ms asynchronous multisensory signals. We compared 1) the sum of a multisensory condition plus null versus the sum of auditory and tactile alone conditions and 2) multisensory conditions to each other, shifted appropriately. We observed multisensory integration effects in both evoked response potentials (ERP) and theta-band phase-locking (similar approach as Senkowski et al., 2007; Exp Brain Res) predominantly for asynchronies +/-20ms and +/-70ms, indicating that auditory and tactile signals are integrated only within a specific temporal window. These effects emerged earlier (100-250ms) over sensory regions and later (250-400ms) over posterior regions. Furthermore, correlations over participants of the RT redundancy effect with the contrast of multisensory to unisensory ERPs were also found, indicating a trait stable over days. Neural indices, and behavioural trait correlates, of a novel auditory/facial-tactile paradigm revealed relevant evoked and phase-locked effects dependent on the critical window for temporal integration. This passive paradigm is well suited to future studies (e.g. asleep, motor/cognitive impairments).

**Disclosures:** J.M. Zumer: None. T.P. White: None. U. Noppeney: None.

**Poster**

**509. Cross-Modal Processing: Temporal Factors**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 509.11/N24
Title: Differential visual modulation of auditory activity in cat A1 between supra- and infragranular layers

Authors: *J. KRUEGER FISTER¹, L. R. KURELA¹, A. R. NIDIFFER², T. A. HACKETT², M. T. WALLACE²; ¹Neurosci., ²Hearing and Speech Sci., Vanderbilt Univ., Nashville, TN

Abstract: Objects and events in our world are frequently specified by more than one sensory modality. Specialized regions within the brain are tasked with actively integrating these multisensory cues in order to facilitate behavior and perception. Traditional association regions in cortex receive convergent inputs from different thalamic and cortical sources and have been intensively studied for their multisensory processing capabilities. However, recent findings implicate that sensory-specific (i.e., unisensory) cortices can also be modulated by information from a different sensory modality. For example, neuronal responses within regions of auditory cortex are influenced by visual and somatosensory stimulation at the level of both spiking and local field potentials (LFP). Here we sought to expand upon these observations by examining visual influences on auditory responses in the primary auditory cortex of the cat (n = 3), with an emphasis on laminar differences that may provide important insights into multisensory modulatory networks. A 16 or 24 channel multilaminar electrode was advanced into primary auditory cortex (A1) and multi-unit (MUA) as well as LFP activity was recorded to visual only, auditory only, and combined audiovisual stimulation while stimulus location varied across azimuth and elevation. Although visual only MUA was rarely encountered, visual LFPs were robust and had similar activity onsets across all layers. Auditory and audiovisual LFPs were also stimulus location dependent and varied between cortical layers. When measuring peak activity, visual LFPs were maximum in infragranular (IG) layers while auditory and audiovisual LFPs peaked in supragranular (SG) and granular (G) layers. Multisensory interactions across the laminae strongly dependent on the magnitude (area under the curve) of the visual influences and the location of the stimuli. When collapsing across all tested elevations, stimuli at central locations elicited reduced audiovisual LFPs when compared to auditory LFPs in the SG layers, while audiovisual LFPs exceeded auditory LFPs in IG layers. With peripheral azimuths, the opposite held true. Findings when collapsing across all azimuths are less clear but suggest a general trend of decreased audiovisual LFPs in SG and increased audiovisual LFPs in IG laminae when contrasted to auditory LFPs. These results indicate that although visual influences into auditory cortex are broad across all layers, they have differential modulatory effects in SG and IG layers dependent upon stimulus location, hinting at potentially distinct processing strategies between networks involving cortical or thalamic loops.
Title: Exploring the role of synchrony in auditory-visual integration in ferrets and humans

Authors: *G. P. JONES, S. M. TOWN, K. C. WOOD, H. ATILGAN, S. DUNN, J. K. BIZLEY;
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Abstract: Animals and humans integrate sensory information over time, and combine this information across modalities in order to make accurate decisions in complex and noisy sensory environments. Multisensory integration occurs at multiple levels within the brain, including within early sensory cortices. However, little is known about the perceptual consequences of early sensory integration in the context of multisensory integration and decision-making. To address this question we adapted a previously published audio-visual decision making task (Raposo, et. al. 2012). This required that subjects (ferrets and humans) accumulate evidence over time in order to estimate the average rate of trains of short auditory and/or visual events. Events were 20 ms white noise bursts or flashes and formed trains lasting 1000 ms. Throughout the event train the instantaneous event rates were varied using fixed “short” and “long” gap lengths (610/120 ms for humans, 50/230 ms for ferrets), meaning the accuracy with which the overall event rate could be estimated increased over time (subjects were unable to respond before the end of the presentation period). Rate discrimination was assessed in both unisensory auditory and visual conditions as well as in synchronous and asynchronous multisensory conditions. In the asynchronous multisensory condition event distributions (for the same rate) were allowed to vary between modalities, and/or a variable offset was added the stimulus onset time between the two modalities. As previously reported for rats and humans, humans’ performance improved in by a similar extent in the multisensory conditions, and was not greatly affected by event distribution asynchrony. In addition relative offsets (-50 to 50 ms) to stimulus onset times did not reduce
performance, and reaction times were significantly faster in the multisensory conditions. Ferrets exhibited a similar pattern of behaviour, with improved discrimination in both multisensory conditions. Trained ferrets were implanted with chronic electrode arrays allowing recording from auditory cortex (AC) during task performance. Given the prevalence of auditory-visual interactions documented in AC of the anesthetised ferret (Bizley et al., 2007) it seems likely that auditory and visual signals might be integrated in early sensory cortex during behaviour. Analysis will focus on how synchronous and asynchronous visual signals influence the representation of auditory events in AC via both spiking and local field potential activity, and whether neural correlates of improved multisensory discrimination can be observed in AC.


Poster

509. Cross-Modal Processing: Temporal Factors

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 509.13/N26

Topic: D.03. Multisensory Systems

Support: ONR-MURI grant N000141010278

NIH-NIDA grant R01DA040990-01

The Hertz Foundation George Lerman Fellowship

Title: Cross-modal interactions rescue temporally suppressed saliency of visual stimuli: A computational model

Authors: *G. GILLARY1,2,3; E. NIEBUR1,2,3;  
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Abstract: In current models, visual saliency is computed independently for each image frame. However, both physiological and psychophysical evidence shows that object representations admit a level of permanence thereby causing interactions across time. For instance, recent psychophysical data (van der Burg et al., J. Exp. Psych HPP 34:1053-65, 2008) demonstrate that visual saliency also has a coarse temporal representation. Search for a visual target that by itself is found efficiently is made inefficient when the target image frame is embedded in a series of frames with salient distractors. If, however, the target frame co-occurs with a salient short tone
(or a short tactile stimulus; van der Burg et al, Neurosci. Lett. 450:60-4, 2009), search for the target becomes efficient. We interpret these results by assuming that the salient stimuli in the frames surrounding the target frame suppress the saliency of the target. Extra-visual stimuli presented simultaneously with the target then increase its salience, making it pop-out from its surround and resulting in efficient search. Here we present a model showing that cross-modal modulation of neural activity in early sensory areas can provide the precise temporal and spatial interaction that is necessary to increase the salience of the target visual stimulus. We do this by filtering each event through two layers, one with a slow and the other with a fast time constant of decay. The saliency read-out occurs in the layer with the longer time constant. Choosing the latter as about 200 ms, compatible with the time course of proto-object representation in extra-striate cortex (O’Herron and von der Heydt, Neuron 5:801-9, 2009), generates interference between subsequent frames, thereby suppressing the target’s inherent saliency. Cross-modal modulation of the short time constant layer, but not the long time constant layer, at the time of target onset rescues its salience. Our model makes two important contributions. First, it extends saliency models (e.g. Itti et al, IEEE-PAMI 11:1254-9, 1998) to include permanence of proto-objects. Second, it shows that interactions between salient auditory or tactile events and early visual areas can provide temporally precise modulation of temporally broad representations in later areas.

Disclosures: G. Gillary: None. E. Niebur: None.

Poster

509. Cross-Modal Processing: Temporal Factors

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 509.14/N27

Topic: D.03. Multisensory Systems

Support: NSERC CIHR

Title: Enhanced tactile perception in musicians

Authors: *S. P. LANDRY, S. PAGE, F. CHAMPOUX;
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Abstract: PROBLEMATIC: The perception of our environment is based on the integration of proximal and distal, or respectively egocentric and allocentric, sensory information. From a
sensory point of view, long-term musical training, which is essentially an enriched multisensory training environment, has been suggested to alter anatomical sensory regions and enhance behavioural sensory abilities. It has also been suggested that long-term musical training could have effects on the integration of multisensory information. However, no study has investigated the effects of musical training on sensory frames of reference. The crossed arm temporal order judgement (TOJ) task has been suggested to measure the role of vision, an allocentric sensory information, on a tactile, an egocentric sensory information, response criterion. OBJECTIVE: The main objective of this study is to determine the effect of long-term musical training on the crossed arm TOJ task. We hypothesise that musicians will be more influenced by information from the egocentric frame of reference in the crossed arm TOJ condition than control group participants. METHODS: 28 participants (14 musicians, 14 controls), matched for age and sex, were recruited for the present study. A TOJ task was used in which a conflict is induced between allocentric and egocentric response criteria. The two groups’ average proportion of correct responses (PCD) scores were compared using a t-test. RESULTS: The performed analysis revealed a significant difference between PCD scores between groups (p = 0.016). DISCUSSION: These results suggest that long-term musical training alters the influence of conflicting information from egocentric and allocentric sensory frames of reference. Musical training, which requires individuals to have an enhanced sense of spatial limb position, appear to place greater importance on limb position in relation to the self, thus enhancing the egocentric frame of reference. Moreover, these results suggest a previously unreported effect of musical training on sensory frames of reference that could be used to explain previously reported altered multisensory ability in musicians.

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Poster

509. Cross-Modal Processing: Temporal Factors

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 509.15/N28

Topic: D.03. Multisensory Systems

Support: BBSRC H5184800

Title: How attention modulates neural excitation and inhibition


Title: Crossmodal perceptual adaptation implies neuronal convergence of auditory and tactile frequency signals

Authors: *L. Crommett, A. Pérez-Bellido, J. M. Yau;
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Abstract: We perceive temporal frequency information by audition and touch. Because these modalities reciprocally influence each other in frequency perception, temporal frequency channels appear to be linked across audition and touch. Auditory and tactile perceptual channels may be tied explicitly if common neural populations support auditory and tactile frequency processing. Adaptation paradigms have been used previously to infer neural tuning properties in psychophysical experiments. In a series of psychophysical experiments, we employed a crossmodal frequency adaptation paradigm to test the hypothesis that a common frequency-tuned neural population processes auditory and tactile frequency signals. Participants (n = 20) each performed a tactile frequency discrimination task in 3 experiment sessions. Each session began with an auditory adaptation period (180s) during which the participant received prolonged auditory stimulation (adaptation conditions with bandpass noise stimuli centered at 200Hz or 400Hz) or silence (control condition). After initial adaptation, participants performed trials of a 2AFC tactile discrimination task in which they judged which of two vibrations presented sequentially to their finger was perceived as being higher in frequency. Vibration frequencies ranged from 100-300Hz. We used a generalized linear mixed effects model (GLMM) to test whether auditory adaptation modulated tactile discrimination performance and whether this modulation was frequency-specific. Crossmodal adaptation significantly improved tactile frequency sensitivity when the spectral composition of the noise adaptor overlapped the tactile test frequencies. We implemented a simple and biologically plausible model that represents tactile frequency information with likelihood functions computed from a population of sensory neurons. By allowing auditory adaptation to modify the model’s sensory neuron response characteristics, our model reproduced the frequency-specific crossmodal aftereffects. These psychophysical and modeling results support the hypothesis that auditory and tactile signals converge on a common frequency-tuned neural population.

Poster

509. Cross-Modal Processing: Temporal Factors

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 509.17/N30

Topic: D.03. Multisensory Systems

Support: Caroline Wiess Law Fund for Research in Molecular Medicine

Title: Decoding modality-specific and modality-invariant temporal frequency representations in the human brain

Authors: A. PEREZ-BELLIDO¹, *K. A. BARNES², M. TOMMERDAHL³, J. M. YAU¹;
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Abstract: We perceive temporal frequency information by audition and touch. Traditional models of sensory cortex function segregate auditory and somatosensory information in modality-specific cortical systems, but temporal frequency processing for audition and touch may not be so independent. Auditory and tactile temporal frequency perception is closely linked and spatially overlapping regions of sensory cortex respond to auditory and tactile stimulation. These results may reflect common auditory and tactile frequency representations that are supported by shared neural circuitry. In the present study we combined functional magnetic resonance imaging (fMRI) and multivariate pattern analysis (MVPA) to characterize auditory and tactile frequency representations. In an event-related fMRI experiment, participants received auditory and tactile stimulation (75, 130, 195, 270 and 355 Hz) in separate scans as they performed an attention-demanding frequency discrimination task. This design enabled us to quantify BOLD signal changes and spatial activation patterns to identical stimulus frequencies presented separately by audition and touch. We trained linear support vector machines to classify stimulus frequency in whole-brain searchlight and ROI-based analyses. We sought to identify modality-specific frequency representations by training and testing our frequency decoder on data acquired from only the auditory or tactile scans (within-modality classification). We sought to identify common frequency representations by training the decoder on data from one modality and testing classification on data from the other modality (across-modality classification). Preliminary analyses indicate robust stimulus frequency decoding performance for both within-modality classification and across-modality classification. These preliminary results reveal modality-specific and modality-invariant temporal frequency representations in the human brain.

Poster
509. Cross-Modal Processing: Temporal Factors

Location: Hall A

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Topic: D.03. Multisensory Systems

Support: NIH Grant DC014114-01

Title: Multisensory temporal processing in the inferior colliculus

Authors: *A. R. NIDIFFER, R. RAMACHANDRAN, M. T. WALLACE; Hearing and Speech Sci., Vanderbilt Univ., Nashville, TN

Abstract: The presence of a cue in one sensory modality can enhance the detection of and the speed of reaction to cues in another modality. Furthermore, the magnitude of this behavioral enhancement has been shown to be dependent on both the intensity and the temporal relationship of the unisensory components of the multisensory stimulus complex. It is well established that these behavioral enhancements occur over a restricted range of stimulus onset asynchronies (SOAs), within the so-called “temporal window of multisensory integration.” Prior neurophysiological work has shown that multisensory enhancement in the superior colliculus is dependent on the temporal relationship between the component unisensory stimuli. Additionally, visual cues have been shown to modulate the responses of auditory signals in the inferior colliculus and even elicit responses when presented alone. Despite this growing evidence for multisensory processing in a traditional component of the ascending auditory pathway, numerous questions remain about the nature of the visual influences on auditory responses in the IC. One of these questions is whether similar temporal constraints exist for these audiovisual interactions in the IC as have been detailed for the SC. To begin to answer this question, monkeys were trained in a focused attention paradigm to report the detection of an auditory cue via lever release. Brief bursts of broadband white noise at varying intensity levels were presented alone and in the presence of an irrelevant, low-intensity visual stimulus at several different SOAs, and responses from IC neurons were recorded and related to behavioral performance. Behaviorally, monkeys showed speeded responses in the presence of the visual cue, but only for a restricted window of SOAs. Auditory neurometric thresholds were found to change in the presence of the visual stimulus, and were typically above concurrently measured psychometric thresholds. Furthermore, visual modulation of auditory responses was found to be dependent upon the
temporal relationship of the irrelevant visual cue in a manner concordant with that found for the SC. Collectively, these results illustrate the relationship between neurometric and psychometric performance for IC neurons and auditory detection, and show that the temporal relationship between the auditory and visual signals in the IC is an important factor in how these signals are integrated.


Poster

509. Cross-Modal Processing: Temporal Factors

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Simons Foundation Explorer Award

Wallace Foundation

Vanderbilt Institute for Clinical and Translational Research (VICTR)

Title: Perceptual training enhances temporal acuity for audiovisual speech

Authors: *M. A. DE NIEAR*¹, P. B. GUPTA², M. T. WALLACE¹;

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Abstract: The synthesis of information from different sensory modalities, termed multisensory integration, has been shown to alter behavior and affect higher order cognitive processes. The integration of auditory and visual cues is a particularly important process for the comprehension of speech. The temporal relationship between the auditory and visual components of speech is critical for their proper integration and binding, and has been characterized by constructs such as the temporal binding window (TBW) i.e., the time interval within which auditory and visual stimuli are highly likely to be integrated or bound). Recently, several studies have examined the plasticity of the TBW and have shown a significant narrowing, but have only focused on low-level multisensory stimuli (visual flashes and auditory beeps). The present study explored whether perceptual training was capable of altering temporal acuity for higher order multisensory stimuli - audiovisual phonemic speech - in typically developed adults. The TBW for phonemic speech and low-level multisensory stimuli (visual flashes and auditory beeps) was measured
using a simultaneity judgment (SJ) task prior to and following feedback training. Training constituted receiving trial-by-trial visual feedback in the form of a green check mark or red X while completing the SJ task during one hour of training on four consecutive days. To control for exposure to phonemic speech stimuli, an additional group of participants completed a passive exposure paradigm that required them to complete an oddball detection task while audiovisual stimuli were presented at identical SOAs for the groups receiving perceptual training. In agreement with previous findings, perceptual training enhanced temporal acuity for low-level stimuli as indexed by a narrowing of the TBW. Novel to this study, perceptual training also enhanced temporal acuity for audiovisual phonemic speech as indexed by a narrowing of the TBW while TBW narrowing was not observed for participants in the exposure group. Changes in temporal acuity observed following perceptual training were exclusive to either speech or low-level stimuli, suggesting that the perceptual training paradigm used by this study did not enhance general temporal acuity for multisensory processes. Future work will explore whether perceptual training for phonemic speech elicits generalized behavioral benefits for speech comprehension. Overall, the results suggest that perceptual training is capable of enhancing temporal acuity for audiovisual speech in adults.


Poster

509. Cross-Modal Processing: Temporal Factors

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Title: Connecting individual differences in sensory and cognitive function in healthy aging

Authors: *S. H. BAUM¹, R. A. STEVENSON², P. A. NEWHOUSE², M. T. WALLACE³; ¹Vanderbilt Brain Inst., ²Psychiatry, ³Hearing and Speech Sci., Vanderbilt Univ., Nashville, TN; ⁴Psychology, Univ. of Toronto, Toronto, ON, Canada
Abstract: Traditional aging research has focused on changes in domains such as sensory function and cognitive performance, with performance declines noted on a number of tasks in both domains. However, little work has been done to link across these seemingly disparate domains. Given that incoming sensory information forms the building blocks for higher order cognitive processing, we hypothesized that individual differences in sensory function would be correlated with measures of cognitive processing. Healthy older adults (ages 45-70) completed a battery of sensory and cognitive tasks across two laboratory visits. The sensory task battery included measures of auditory temporal acuity (auditory Temporal Order Judgment [TOJ]), visual temporal acuity (visual TOJ), as well as a series of measures of audiovisual (AV) integration, including an AV TOJ with both flash/beep and speech stimuli, Simultaneity Judgment (SJ) with both flash/beep and speech stimuli, the sound induced flash illusion (SIFI), and the McGurk illusion. The cognitive task battery included Critical Flicker Fusion (CFF), Choice Reaction Time (CRT), the Connors Continuous Performance Task (CPT), and the Buschke Selective Reminding Task (SRT). We found significant correlations within (e.g. temporal acuity on the flash/beep SJ task with temporal acuity on the speech SJ task for both auditory leading: $r = 0.75, p = 0.03$ and visual leading asynchronies: $r = 0.88, p = 0.004$) and across (e.g. greater reaction time variability in the CPT with decreased susceptibility to the SIFI illusion, $r = -0.92, p = 0.02$) domains (Figure 1A). A hierarchical clustering analysis (Figure 1B) revealed both expected connections (e.g. clustering of the two AV illusions) but also interesting connections across domains (e.g. temporal acuity in the flash/beep TOJ task and relevant intrusions in the SRT). A rich characterization of both sensory and cognitive function may be able to create a unique profile for healthy older adults, which can be compared to clinical populations to determine what kinds of changes might be precursors to different aging pathologies.


Poster

509. Cross-Modal Processing: Temporal Factors

Location: Hall A
Title: Speeded microstate transitions are associated with multisensory perceptual segregation

Authors: *D. M. SIMON, A. THELEN, M. T. WALLACE;
Vanderbilt Univ., Nashville, TN

Abstract: Multisensory integration is the process of combining information from multiple sensory modalities and has been shown to confer numerous behavioral and perceptual benefits. A primary determinant of multisensory interactions is the temporal relationship between paired sensory inputs, and the temporal binding window (TBW) is a probabilistic construct representing the interval of time in which there is a high likelihood of events occurring in close temporal proximity of being fused into a single perceptual event. The overall size of the TBW serves as a measure of multisensory temporal acuity, and large individual differences have been reported across studies. This indicates that the ability to segregate multisensory stimuli that are occurring in close temporal proximity into separate events is highly variable. Here we investigated the neural correlates of such perceptual segregation both within and between subjects using audiovisual stimuli and high density EEG recording. Whole brain topographic analysis revealed that differences in neural generator configuration during perceptual processing corresponded with both individual ability to segregate stimuli and perceptual outcomes. Individuals with narrow TBWs, and thus high ability to segregate stimuli with small temporal offsets into two events, had faster transitions to a sequentially second functional microstate. Within subject comparisons further indicated that faster transitions to a sequentially second functional microstate were associated with perceptual segregation for both auditory leading and visual leading stimuli. Further, overall duration of the second microstate was longer in individuals with superior temporal acuity and when stimuli were perceived as occurring separately. These results provide evidence that the latency of neural generator recruitment corresponds with perceptual outcomes during a multisensory temporal task.

Title: Multisensory processing within thalamocortical networks occurs by temporal coupling of neuronal firing and oscillatory activity

Authors: *M. BIENER, N. CICHON, K. SIEBEN, I. L. HANGANU-OPATZ; Developmental Neurophysiology, Inst. of Neuroanatomy, Univ. Med. Ctr. Hamburg-Eppendorf, Hamburg, Germany

Abstract: Behavioral performance relies on optimal integration of information from different sensory modalities. Phase reset and power modulation of oscillatory activity have been identified as mechanisms underlying the integration of sensory inputs within interconnected primary somatosensory (S1) and visual (V1) cortices. However, the contribution of individual neuronal firing in these areas to multisensory interactions remains largely unknown. Moreover, the fast timing of these interactions suggests that already subcortical areas on the sensory tract, such as thalamic nuclei, might converge inputs from different sensory modalities. Here we assess the impact of uni- (light flash or whisker deflection) and bimodal stimulation (i.e. simultaneous light flash and whisker deflection) on neuronal firing and oscillatory activity within thalamocortical networks by performing simultaneous extracellular recordings from S1 and V1 as well as the corresponding thalamic nuclei, ventral posteromedial nucleus (VPM) and lateral geniculate nucleus (LGN), in juvenile rats in vivo. The identity of spiking neurons was assessed by feature-based clustering analysis. After bimodal stimulation the firing of pyramidal neurons in S1 significantly decreased when compared to unimodal stimulation. While both uni- and bimodal stimuli phase-locked the firing of pyramidal neurons in S1 and V1 to the corresponding gamma activity, the coupling between the firing in one primary cortex and theta-alpha activity in the other primary cortex was strengthened only after bimodal stimulation. Moreover, the preferred phase of firing for S1 neurons switched from the trough of network oscillations in V1 after unimodal stimulation to the peak of V1 network oscillations when bimodal stimuli were applied. Bimodal stimulation strongly modulated the neuronal firing of thalamic nuclei VPM and LGN. In line with their position on the sensory tract, the firing to bimodal stimulation in both thalamic nuclei occurred at a shorter latency when compared to S1 or V1. In contrast to the primary sensory cortices, thalamic firing was enhanced by bimodal stimuli. Taken together, temporal coupling of individual neuronal firing and oscillating population activity provides the framework for cross-modal processing within thalamocortical networks.
Disclosures: M. Bieler: None. N. Cichon: None. K. Sieben: None. I.L. Hangan-Opatz: None.

Poster

509. Cross-Modal Processing: Temporal Factors

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 509.23/N36

Topic: D.03. Multisensory Systems

Support: Caroline Wiess Law Fund for Research in Molecular Medicine

Title: fMRI adaptation reveals population tuning for tactile and auditory stimulus frequency in human cortex

Authors: K. A. BARNES¹, M. TOMMERDAHL², *J. M. YAU¹;
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Abstract: We perceive temporal frequency information by audition and touch. Traditional models of sensory cortex function segregate auditory and somatosensory information in modality-specific cortical systems, but temporal frequency processing for audition and touch may not be so independent. Auditory and tactile temporal frequency perception is closely linked and spatially overlapping regions of sensory cortex respond to auditory and tactile stimulation. These results may reflect interactions between distinct neural populations that independently represent auditory and tactile frequency information. Alternatively, a subpopulation of frequency-tuned neurons could represent both auditory and tactile information. Here, we used fMRI adaptation to characterize population neural tuning for auditory and tactile temporal frequency in human cortex. This method exploits the tendency for sensory neural responses to be attenuated following repeated stimulation to infer voxel-level population tuning preferences. In an event-related fMRI experiment, participants received auditory and tactile stimulation as they performed a frequency discrimination task. Each trial comprised a series of 3 stimuli (adaptor stimuli; always matching in frequency) followed by a single (probe) stimulus. Adaptor and probe stimuli could be auditory or tactile. Stimulus frequency could be 100Hz or 300Hz. To test for frequency tuning, we compared BOLD responses from trials in which the frequencies of the adaptor and probe stimuli matched (SAME) with responses from trials in which the frequencies differed (DIFF): smaller responses for SAME trials likely reflect frequency-specific neural adaptation. From analysis of trials in which adaptor and probe modalities matched (auditory-auditory or tactile-tactile trials), we sought to identify voxels exhibiting modality-specific frequency tuning. From analysis of trials in which adaptor and probe modalities differed
(auditory-tactile or tactile-auditory trials), we sought to identify voxels exhibiting modality-invariant frequency tuning. Notably, crossmodal BOLD adaptation reveals convergence of auditory and tactile frequency signals on a common neural population. Preliminary results reveal the existence of modality-specific and modality-invariant frequency tuning mechanisms.


Poster

510. Striate Cortex Circuitry

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 510.01/N37

Topic: D.04. Vision

Support: NIH Grant EY020679

Title: Phase selectivity of simple and complex cells in visual cortex

Authors: *C. PONS\textsuperscript{1}, M. JANSEN\textsuperscript{1}, X. LI\textsuperscript{1}, Y. BERESHPOLDOVA\textsuperscript{2}, H. SWADLOW\textsuperscript{2,1}, J. ALONSO\textsuperscript{1,2};
\textsuperscript{1}Biol. Sci., State Univ. of New York, New York, NY; \textsuperscript{2}Psychology, Univ. of Connecticut, Storrs, CT

Abstract: Neurons in primary visual cortex (area V1) are classified into simple and complex cells based on their linearity of spatial summation (Hubel and Wiesel, 1962; Movshon et al., 1978). It is commonly assumed that simple cells are more phase selective than complex cells. However, the relationship between linearity of spatial summation and phase selectivity has not been systematically measured. In order to address this question, we measured the linearity of spatial summation and phase selectivity of 189 V1 single neurons (90 simple, 99 complex) recorded in two awake rhesus monkeys. Linearity of spatial summation was measured with drifting gratings as the response amplitude at the frequency of the grating (F1) divided by the mean firing rate (F0). Phase selectivity was measured as the phase bandwidth from responses to static gratings (10 different phases, each presented for 500 msec and separated by 500 msec blanks). The phase bandwidth (PB) was measured at half-maximum response directly from the raw data (PBr) or from a von Mises fit (PBf, $R^2 > 0.7$). Our results demonstrate that linearity of spatial summation is correlated with phase selectivity in the entire cell population (PBr: $r = -0.62$, $p < 0.0001$, $n = 189$; PBf: $r = -0.51$, $p < 0.0001$, $n = 131$) and in simple cells (PBr: $r = -0.47$, $p < 0.0001$, $n = 90$; PBf: $r = -0.46$, $p < 0.0001$, $n = 78$) but not in complex cells (PBr: $r = -0.18$, $p = 0.075$, $n = 99$; PBf: $r = -0.04$, $p = 0.765$, $n = 53$). Correlation values were slightly
stronger when measured from responses evoked when the grating was turned on than when it
was turned off (e.g. PBr: $r = -0.62$ versus $-0.55$ for entire cell population and $r = -0.47$ versus -
0.31 for simple cells). As expected, the mean phase bandwidth was significantly larger for
complex cells ($0.74 \pm 0.27$ cycles for $F_1/F_0 < 0.8$) than simple cells ($0.43 \pm 0.27$ cycles for
$F_1/F_0 > 1.2$, $p < 0.00001$, Wilcoxon test). However, the range of phase bandwidths was
surprisingly broad in both (PBr: $0.18$-1 versus $0.27$-1 cycles, PBf: $0.16$-1 versus $0.17$-1 cycles),
even if we selected only cells at the borders of the $F_1/F_0$ distribution ($F_1/F_0 > 1.5$ versus $F_1/F_0$
$< 0.5$, PBr: $0.18$-1 versus $0.27$-1, PBf: $0.17$-0.70 versus $0.27$-1). The distribution of phase
bandwidth for the largest $F_1/F_0$ values (simple cells) was best described by a Gaussian function
(e.g. PBr for $F_1/F_0 > 1.5$, $R^2 = 0.84$, $n = 31$; PBf for $F_1/F_0 > 1.2$, $R^2 = 0.91$, $n = 62$) and the
distribution for the lowest $F_1/F_0$ values (complex cells) was best described by an exponential
function (e.g. PBr for $F_1/F_0 < 0.5$, $R^2 = 0.98$, $n = 29$; PBf for $F_1/F_0 < 0.2$, $R^2 = 0.66$, $n = 45$).
These results indicate that, although complex cells show less phase selectivity than simple cells,
both simple cells and complex cells are robustly tuned for spatial phase.

Disclosures: C. Pons: None. M. Jansen: None. X. Li: None. Y. Bereshpolova: None. H.
Swadlow: None. J. Alonso: None.

Poster

510. Striate Cortex Circuitry

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 510.02/N38

Topic: D.04. Vision

Support: NIH Grant EY02067901

NIH Grant EY007556

DFG Research Fellowship (KR 4062/1-1)

Title: Changes in the balance of ON and OFF cortical responses with the spatial-frequency
content of the visual scene

Authors: *M. Jansen*<sup>1</sup>, X. Li<sup>2</sup>, R. Lashgari<sup>2,4</sup>, J. Kremkow<sup>2</sup>, Y. Bereshpolova<sup>3</sup>,
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Abstract: We have previously demonstrated that low spatial frequencies generate stronger OFF than ON responses in cat visual cortex and that this OFF-dominance is reduced as the spatial frequency increases (Kremkow 2014). Here, we demonstrate that the spatial-frequency dependency of cortical OFF dominance is also present in the visual cortex of awake behaving primates and we investigate its possible neuronal mechanisms. We recorded from 235 single neurons from the primary visual cortex of two awake macaques using a chronically implanted, ultrathin, multi-electrode array (Swadlow 2005; Chen et al., 2008). We mapped the receptive fields by spike-trigger-averaging gratings (Ringach et al. 1997) that were presented in a rapid sequence (80 Hz) with 88 different orientations, 41 spatial frequencies and 4 phases. The gratings had four different sizes that were scaled so that a decrease in size by 2, 4 and 8 times resulted in an increase in spatial frequency content by the same factor. Measurements of preferred spatial phase with flashed gratings optimized for the cell’s stimulus preferences (orientation, spatial frequency and size) revealed a slight bias towards OFF-dominated phases (65% in 110 comparisons between even phases measured within ± 1/8 of a phase cycle). This OFF-dominance became very pronounced when we mapped receptive fields with low spatial frequencies (79% of 235 neurons) and was only reduced to 50% when we increased the spatial frequency content of the stimuli by reducing the grating size to 1/8th. The ratio between the number of ON and OFF dominated receptive fields mapped with flashed gratings was strongly correlated with grating size (R²=0.99, n=4 sizes), a correlation that could be explained by two possible mechanisms: changes in ON/OFF balance within the receptive field of each neuron or changes in the relative number of ON and OFF dominated neurons driven with flashed gratings. To distinguish between these mechanisms, we measured changes the balance of ON and OFF responses across stimulus conditions for each cell and found that the changes were modest but significant (reducing grating size by half changed ON/OFF balance from 0.67 to 0.87, p=0.005, paired t-test). Moreover, increasing the spatial frequency of a drifting grating caused an average shift in phase (-0.12 ± 0.07) that was ~0.1 cycles greater than the average shift expected from changes in response latency (e.g. -0.02 ± 0.02 for contrast and 0.04 ± 0.02 for size). We conclude that the spatial-frequency dependency of cortical OFF dominance is generated by both changes in the relative numbers of ON and OFF-dominated neurons and small changes in the ON/OFF response balance within each single neuron.

Disclosures: M. Jansen: None. X. Li: None. R. Lashgari: None. J. Kremkow: None. Y. Bereshpolova: None. H. Swadlow: None. Q. Zaidi: None. J. Alonso: None.

Poster

510. Striate Cortex Circuitry

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM
Program/#Poster#: 510.03/N39

Topic: D.04. Vision

Support: Max Planck Society

ERC (GL)
HFSP (JF)

Title: Classification of GABAergic interneurons in turtle visual cortex

Authors: *C. M. MUELLER, J. FOURNIER, G. LAURENT;
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Abstract: Information processing in sensory cortices relies on balanced interactions between and within populations of excitatory and inhibitory neurons. In turtle, the primary cortical recipient of geniculate afferents, the three-layered dorsal cortex, is in a position analogous to that of primary visual cortex (V1) in mammals. In the present study we defined the outlines and extent of turtle (Trachemys scripta) visual cortex by transneuronal tracing from the eye, and estimated the fraction of inhibitory neurons by staining for GABA and the pan-neuronal marker NeuN. Finally, using immunostaining for a variety of known markers of mammalian GABAergic neuronal subtypes, we examined the degree of heterogeneity among interneurons. Transneuronal tracing using intra-ocular injection of wheat germ agglutinin conjugated to horseradish peroxidase (WGA-HRP) revealed cortical labeling restricted to superficial layer I of the contralateral cortex. Labeling extended from the rhinal fissure to the cortical midline in the rostral third of the telencephalon. The neuronal marker NeuN labeled an average of about 26,000 neurons per mm$^2$ of visuo-recipient cortical area, of which only 1,700 cells/mm$^2$ (~7%) was immunoreactive to GABA - a fraction smaller than in mammalian V1. Double-immunostaining with antibodies directed against GABA and individual markers co-expressed with GABA in mammalian cortex revealed four antigens present in a substantial fraction of GABA$^+$ neurons: NPY (16%), somatostatin (18%), calbindin (28%), and the ionotropic 5HT-receptor (5HT3aR, 31%). All four antigens were mainly found in the lower half of the cortical thickness (layers II, III, and lower layer I). Additional antigens (e.g. calretinin and nitric oxide synthase) were found in minor fractions (<1%) of cortical GABAergic cells. Immunopositivity to parvalbumin and choline acetyl transferase was entirely absent in turtle visual cortex, despite reliable staining in subcortical tissue. Double staining revealed a full overlap of NPY with somatostatin expression. Triple-labeling with antisera against somatostatin, calbindin, and 5HT3aR revealed widespread co-expression of two or all three of these markers in individual neurons. About half (53%) of the interneuron population expressed no co-marker. GABAergic neuron heterogeneity in turtle visual cortex, assessed by immunochemical co-markers, thus appears to be less than in mammalian cortex. Parallel experiments are now testing the possible co-segregation of interneurons into physiological, connectional, and functional subtypes.

Poster

510. Striate Cortex Circuitry

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 510.04/N40

Topic: D.04. Vision

Support: R21NS088906-01

New York Structural Biology Center

P30EY013079-15

Title: Estimating synaptic density using 3D FIB/SEM and 3D confocal microscopy of LGN afferents in monkey V1

Authors: *V. GARCIA-MARIN, M. J. HAWKEN;
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Abstract: The main thalamocortical (TC) recipient layers in primate primary visual cortex (V1) are the two sublayers of 4C, 4Ca and 4Cb, with additional input to layer 6, layer 4A, patches in layer 2/3 and layer 1. A number of studies have focused on estimating the number of synapses made by terminals of TC afferents from the Lateral Geniculate Nucleus (LGN) in layer 4C using a range of different techniques, such as: tracing studies, degeneration techniques, or immunohistochemistry. These studies have provided a wide range of estimates of the TC contribution to layer 4C, from 1.3% to 32% depending on species, sublayer, and microzones, with a median of around 8%. Recently in a 2D transmission electron microscopic (TEM) study using an antibody against the Vesicular Glutamate Transporter 2 (VGluT2) to label the LGN TC afferents we found that in layer 4Cb, VGluT2 accounted for around 16% of the total synapses, while the density in layer 4Ca was 8% of the total synapses. However, recent studies using 3D EM reconstructions indicate the total number of synapses have been underestimated in 2D EM studies. We confirmed these findings using 3D Focussed Ion Beam/Scanning Electron Microscopy (FIB/SEM), showing that quantification in 2D TEM was an underestimation of the total number of synapses by a factor of 2 - 2.5. Therefore although some of the previous studies have provided a relatively reliable estimation of the proportion the TC population contributes to the total synaptic population, they have not accurately estimated the total number synapses or the subset that are TC. To obtain estimates of synapse density in relatively large regions of cortex we
used confocal fluorescent microscopy and 3D reconstruction to estimate the number and volume of VGluT2-ir terminals in layer 4Cβ and in conjunction with estimates of the number of postsynaptic densities (PSD’s) per terminal from 3D EM reconstructions we estimated the total number of TC synapses. We found a close match between the numbers of terminals estimated from EM and those using 3D reconstruction of confocal images. Using these methods allows for systematic studies of the populations of identified synaptic terminal populations across different layers, in different regions and between animals. In addition, obtaining accurate estimates of the total number of synaptic populations is fundamental to establishing the realistic models of brain circuits.

Disclosures: V. Garcia-Marín: None. M.J. Hawken: None.
groups of neurons in the neocortex (Yassin et al., Neuron, 2010; Jouhanneau et al., Neuron, 2014). In this study, we observed strong c-Fos expression sparsely throughout the primary visual cortex under home-cage conditions. To evaluate the spatial distribution of c-Fos expression, we introduced energy-like and entropy-like parameters. These analyses did not require arbitrary thresholds on the c-Fos positivity. Instead we utilized the c-Fos intensity as an analogue signal, without separating them into c-Fos positive or negative neurons. Using geometric energy analysis, we found that strongly c-Fos expressing neurons were spatially clustered in each cortical layer except for layer 1. Using geometric entropy analysis, we were able to determine the mean size of these clusters. The cluster size measured approximately 100 µm in diameter and was significantly smaller in layer 2/3 than in layers 5 and 6.

Disclosures: K. Makino: None. K. Funayama: None. Y. Ikegaya: None.

Poster

510. Striate Cortex Circuitry

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 510.06/N42

Topic: D.04. Vision

Support: Simons Foundation to the Simons Center for the Social Brain at MIT

Title: Advancing a biomarker of reduced GABAergic action in the autistic brain

Authors: *C. E. ROBERTSON¹, A. MYNICK², S. RAJA², E.-M. RATAI³, N. KANWISHER²; ¹Harvard Society of Fellows, Cambridge, MA; ²McGovern Inst. for Brain Research, MIT, Cambridge, MA; ³Martinos Imaging Center, MGH, Charlestown, MA

Abstract: Intro: The primary inhibitory neurotransmitter in visual cortex, GABA, has been linked to autism etiology in animal studies as well as genetic and post-mortem work in humans. Specifically, GABAergic action is posited to be reduced in the autistic brain, producing an overabundance of excitatory neurotransmission. Yet, perturbations in GABAergic signaling have never been experimentally associated with behavioral symptoms in people with autism. We recently demonstrated a striking autistic deficit in binocular rivalry (Robertson et al., 2013), a visual behavior that is governed by excitatory/inhibitory dynamics in the brain. Here, we tested whether the dynamics of binocular rivalry are correlated with levels of excitatory (glutamate) and inhibitory (GABA) neurotransmitters measured \textit{in vivo}. Methods: In 41 participants (21 ASD, age and IQ matched to 20 TDs), we measured binocular rivalry dynamics, the concentration of excitatory (Glutamate) and inhibitory (GABA) neurotransmitters in the primary
visual (test) and primary motor (control) areas of each individual’s brain using Magnetic Resonance Spectroscopy (MRS), and autistic symptom severity (ADOS). Behavioral Results: We replicate our original finding of atypical rivalry dynamics in ASD. Individuals with ASD demonstrate slower rivalry dynamics than controls ($p < 0.001$), with a reduced proportion of fully suppressed percepts ($p < 0.01$). These findings strongly predicted autistic symptoms ($\rho = -0.69, p < 0.003$). Imaging Results: As predicted from models of binocular rivalry, the average concentration of GABA and Glutamate predicted binocular rivalry dynamics across both groups, predicting the proportion of perceptual suppression ($\rho = 0.390, p < 0.014$). Critically, these neurotransmitters exerted differential impact on binocular rivalry dynamics in ASD and Controls: GABA in V1 strongly predicted the dynamics of binocular rivalry in Controls ($\rho = -0.61, p < 0.009$), but demonstrated no impact on rivalry dynamics in the ASD group ($\rho = 0, p = 1$). Conversely, Glutamate predicted rivalry dynamics in both individuals with ($\rho = 0.64, p < 0.005$) and without ASD ($\rho = 0.38, p < 0.1$), as well as autistic symptomatology ($\rho = -0.51, p < 0.026$). These results were specific to V1: GABA concentration in M1 did not predict rivalry dynamics ($p > 0.19$).

Conclusions: Here, we demonstrate a robust disturbance in a fundamental visual phenomenon in individuals with ASD, which strongly predicts GABA concentration. These data validate computational models of binocular rivalry, and mark the first experimental demonstration of a link between a robust autistic symptom and a prominent theory of autistic neural circuitry.

Disclosures: C.E. Robertson: None. A. Mynick: None. S. Raja: None. E. Ratai: None. N. Kanwisher: None.

Poster

510. Striate Cortex Circuitry

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

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Topic: D.04. Vision

Support: Wellcome Trust Grant 095669

Wellcome Trust Grant 095668

Simons Collaboration on the Global Brain grant 325512

Title: Probing excitatory/inhibitory dynamics in awake visual cortex
Abstract: Cortical activity arises from the interplay of recurrently connected excitatory (E) and inhibitory (I) neurons. While theoretical models of E-I interactions have existed for many years (e.g., Wilson and Cowan, 1972), quantitative experimental analysis of E-I dynamics has become possible only recently, thanks to developments in population recording and optogenetics. To study and manipulate E-I dynamics in a quantitative manner, we combined electrophysiological recording using multisite silicon probes with optogenetic stimulation of pyramidal and parvalbumin-expressing (Pvalb) neurons in primary visual cortex (V1) of quietly awake mice. To separately stimulate excitatory and inhibitory populations we used multi-wavelength optogenetics. We crossed the Thy18 mouse line (which expresses ChR2 in a subpopulation of excitatory neurons) with a Pvalb-Cre driver line, and we expressed the red-shifted opsin C1V1 in Pvalb neurons by injecting a conditional virus. Brief blue (445 nm) and green (561 nm) laser pulses were delivered to stimulate the excitatory and Pvalb populations. We summarized the network activity at each moment by two population rates, representing the total firing rates of all recorded wide-spiking (presumed to be mainly excitatory) and narrow-spiking (putative Pvalb) neurons. We derived these population rates directly from the recorded data via a novel analysis technique that did not require spike sorting: for each spike, we used locality-sensitive hashing to estimate a smooth waveform whose width could then be measured accurately. A single blue-light pulse caused an initial brief activation in the excitatory population, which in turn excited the putative Pvalb population, followed by prolonged suppression of both populations. Responsiveness to a second pulse was suppressed to various degrees depending on the interpulse interval. Similarly, a single green-light pulse reliably increased the population activity of putative Pvalb neurons, followed by a less noticeable quiet period. We are currently developing a dynamical system model to predict single-trial responses as a function of spontaneous activity preceding the stimulation. These initial results indicate that in V1 of quietly awake mice, repeated delivery of single laser pulses or pairs of pulses at controlled intervals is effective in probing the dynamics of the network. It may thus be possible to predict the dynamics of excitatory and Pvalb neuronal populations quantitatively on a trial-by-trial basis.

Topic: D.04. Vision

Support: NIH grant EY024946

Title: Thalamocortical input to putative fast-spark interneurons in layer 4 of rabbit visual cortex

Authors: *X. HEI¹, Y. BERESHPOLOVA¹, C. R. STOEZEL¹, J.-M. ALONSO², H. A. SWADLOW¹;
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Abstract: In somatosensory barrel cortex of the rabbit, putative fast-spark inhibitory interneurons in layer 4 (suspected inhibitory interneurons, SINs) receive strong and highly convergent/divergent inputs from neurons in the topographically aligned ventrobasal thalamic barreloid (Swadlow, 1995; Swadlow and Gusev, 2002). We have recently shown that SINs in layer 4 of rabbit visual cortex receive strong input from the retinotopically aligned region of the lateral geniculate nucleus (LGN, Zhuang et al., 2013). However, the rules governing thalamocortical connectivity onto layer 4 SINs in the visual cortex are not known. Here, we simultaneously recorded, in awake rabbits, spontaneous spike trains of concentric neurons in the LGN and SINs in the retinotopically aligned region of layer 4. Cross correlation analyses show that, like SINs in S1, most layer 4 SINs in V1 exhibit sharp peaks in the cross correlograms (indicative of monosynaptic connectivity) at appropriate intervals following the thalamic spikes when LGN and SIN receptive fields are very well aligned. Our preliminary data indicates that the connection probability/strength does not depend on matching either (a) the sign of the preferred contrast of the LGN cell and the layer 4 SIN (on-center or off-center in the LGN, on-dominated vs. off-dominated in the SINs), or (b) the Sustained/Transient response classification of the two cells. Thus, our results suggest that SINs of V1, like those of S1, receive a highly divergent/convergent input from multiple thalamic cell types in the topographically aligned region of their associated specific thalamic nucleus.


Poster

510. Striate Cortex Circuitry

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 510.09/N45

Topic: D.04. Vision
**Title:** Acetylcholine drives cortical microcircuit and modulates temporal dynamics in V1

**Authors:** *H. SUGIHARA*¹, N. CHEN¹²³, M. SUR¹;

**Abstract:** Acetylcholine (ACh) modulates cortical functions including information processing and plasticity. To understand the physiological basis of these functions, it is critical to identify the cortical circuit elements involved. We have previously shown that cholinergic activation of astrocytes and their facilitatory influences on pyramidal neurons (PYR) are crucial to induce plasticity (Chen N, Sugihara H et al., PNAS 2012). In this work, we aim to dissect the neural circuit involved in cholinergic modulation of sensory processing. Specifically, we focus on the temporal dynamics of cortical activity: decorrelation of neuronal responses and desynchronization of local field potential (LFP) using L2/3 mouse primary visual cortex (V1) as a model. Recent studies suggest that inhibitory neurons are important for mediating temporal changes in neural activity. Candidate neurons include regular-spiking inhibitory neurons: somatostatin-expressing (SOM), vasoactive intestinal peptide-expressing (VIP) and layer 1 (L1) neurons. We recorded the cholinergic responses of these inhibitory neuronal subtypes in slice preparations. ACh induced concentration-specific responses in these neurons: SOM neurons were activated by a range of ACh concentrations while VIP/L1 neurons were activated only at high concentration. We further show that this is likely due to the active shaping of inhibitory neuronal responses through defined inhibitory connections between them: SOM neurons inhibit VIP/L1 neurons and this counters the ACh-induced facilitatory responses in the VIP/L1 neurons. In addition, we show that ACh-activated SOM (but not VIP/L1) induced inhibitory currents in parvalbumin-expressing (PV) and PYR neurons. This suggests the presence of an ACh-activated neural circuit comprising direct SOM-PYR and indirect SOM-PV-PYR connections. We next tested the causal relationship between this SOM-driven circuit and decorrelation/desynchronization through hyperpolarizing Arch-expressing SOM neurons. Indeed, hyperpolarization of SOM neurons blocked the cholinergic-mediated desynchronization/decorrelation. Hyperpolarization of VIP neurons did not affect the LFP.
desynchronization. Finally, we stimulated SOM neurons directly by expressing ChR2 in these neurons. Photostimulation of SOM neurons, in the absence of cholinergic stimulation, induced LFP desynchronization. This suggests that direct activation of SOM-driven circuit is sufficient to change temporal dynamics of V1. Collectively, these findings reveal the powerful role of SOM neurons in dynamically shaping the temporal pattern of cholinergic-mediated responses.

Disclosures: H. Sugihara: None. N. Chen: None. M. Sur: None.

Poster

510. Striate Cortex Circuitry

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 510.10/N46

Topic: D.04. Vision

Key Construction Program of the National "985" Project, China

Title: What does cytochrome oxidase histochemistry truly represent in the visual cortex?

Authors: *T. TAKAHATA1,2,
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Abstract: Since late 1970’s, cytochrome oxidase (CO) histochemistry has been widely used for histological analysis to reveal cytoarchitecture of mammalian brains. Particularly in the primate visual cortex, CO staining is useful to identify layers, sublayers and other functional compartments, including CO blobs/patches in the primary visual cortex (V1) and thick/thin stripes in the secondary visual cortex (V2), in otherwise obviously uniform structure. It has been suggested that the enzymatic activity of CO reflects metabolic activity in mitochondria, especially because CO staining intensity drops in the ocular dominance columns (ODCs) for the deprived eye after eye occlusion or removal. Thus, CO staining pattern has been supposed to show map of neuronal activity. Here, I pose a question to this view. First, in any cortical area of any mammalian species, CO staining pattern is fairly similar to the staining of immunohistochemistry for vesicular glutamate transporter 2 (VGLUT2), which has already been confirmed to visualize thalamo-cortical axon terminals. In fact, I have observed that VGLUT2-immunoreactivity (ir) also dramatically decreases in ODCs of V1 after monocular deprivation in the same manner as CO staining, although VGLUT2-ir does not represent neuronal activity of cortical neurons. Second, CO staining pattern does not match to the expression patterns of immediate-early genes, c-Fos, Zif268 and Arc, which have also been suggested to visualize
activity of neurons. Third, CO activity is rarely seen in cell bodies but seen in fibers in the cortex. Therefore, we suggest a possibility that CO staining pattern indeed shows difference in metabolic activity between thalamo-cortical fibers and cortical neurons in the cortex, or strong metabolic activity of distal dendrite of cortical neurons that directly receive inputs from thalamus. In either case, CO staining may show connectivity, but not activity in the cortex. If this is true, the paradigm change will urge us to review the interpretation of CO blobs in V1 and CO stripes in V2: They may represent distribution of thalamo-cortical connectivity, not portions with strong activity of cortical neurons. In addition, interpretation of studies with CO staining for development, cross-species comparison and any disease/transgenic models should be reconsidered.

**Disclosures:** T. Takahata: None.

**Poster**

510. Striate Cortex Circuitry

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 510.11/N47

**Topic:** D.04. Vision

**Support:** General Researcher Program (#2013058415) of National Research Foundation of Korea

**Title:** A developmental model of salt-and-pepper type orientation map in visual cortex

**Authors:** *C. LEE, J. JANG, S.-B. PAIK;
KAIST, Daejeon, Korea, Republic of

**Abstract:** Orientation map in mammalian visual cortex is one of the most studied functional architectures in brain. It has been observed that higher mammals have well-organized quasi-periodic orientation maps, and recently a model study showed that a moiré interference pattern between ON and OFF retinal ganglion cell (RGC) mosaics can seed a consistent spatial periodicity in the maps (Paik & Ringach, 2011). On the other hand, in rodents visual cortex, smooth and periodic map structures are not found, but what is observed is “salt-and-pepper” type structure where preferred orientations of neurons are scattered randomly (Ohki et al., 2005). Despite extensive studies, developmental mechanism of this structure has not been completely understood yet. Here in our model study, we show that the salt-and-pepper type maps can be seeded by a conditional statistical feedforward wiring between the RGC mosaics structure and primary visual cortex (V1). In the developmental model of ON and OFF RGC mosaics, we
assumed local repulsive interactions between the same and the different types of RGCs. To find the wiring rule between retina and V1 that can induce a salt-and-pepper map, we tried two statistical wiring models: (1) sparse wiring model where a small set of RGCs in local sampling area is selected to be wired with the probability of connection as a function of the distance from the V1 cell, and (2) complete wiring model where a V1 cell is connected to every RGC in sampling area with weighted connection strength. In both models, we simulated the wiring with various sampling ranges and examined the orientation selectivity of each V1 cell and the spatial organization of them. We observed that salt-and-pepper map is developed in sparse wiring model with a long sampling range, while a smooth map is achieved as the wiring becomes less sparse, close to complete wiring and the sampling area becomes smaller. Our result suggests that the salt-and-pepper organization of rodents orientation map can be seeded by specific feedforward wiring rule between RGC mosaics and V1. References 1. Paik, S.-B., & Ringach, D. L. (2011). Retinal origin of orientation maps in visual cortex. Nature Neuroscience, 14(7), 919-925. 2. Ohki, K., Chung, S., Ch'ng, Y. H., Kara, P., & Reid, R. C. (2005). Functional imaging with cellular resolution reveals precise micro-architecture in visual cortex. Nature, 433(7026), 597-603.

Disclosures:  C. Lee: None. J. Jang: None. S. Paik: None.
Abstract: Neuronal tuning defined by the level of selectivity to a specific stimulus is one of the hallmarks of cortical computation. Understanding the role of GABAergic interneurons in shaping cortical tuning is rapidly increasing with the ability to specifically manipulate them. Here we show that a class of interneurons that express vasoactive intestinal polypeptide (VIP) shapes the orientation tuning of neurons in mouse primary visual cortex. By combining two-photon imaging with bidirectional optogenetic and chemogenetic manipulation we found that VIP+ cells control the network selectivity to different stimuli. The effect indicates a dual action of VIP+ cells on the network, controlling the specific and non-specific responses, thus contributing to maintaining the variance, or signal-to-noise, across stimuli. These results reveal an important role of inhibition in cortical computations and strongly suggest that orientation selectivity does not merely arise from the projection of excitatory afferents into the cortex but rather is an emergent computation that is dynamically modulated via specific forms of intracortical inhibition.


Poster

510. Striate Cortex Circuitry

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#|Poster#: 510.13/O1

Topic: D.04. Vision

Support: Leibniz Award of the DFG

Neurocure Stipend

Title: Polyploidy shapes the cellular and areal diversity of cortical layer 5

Authors: *M. BRECHT*¹, J. SIGL-GLOECKNER²;
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Abstract: Principal cell diversity is a central, yet unresolved issue in the neurobiology of the cerebral cortex. We address this issue in rat cortex by analyzing soma / nucleus size on the one hand, and assessing DNA content and nuclear organization by DAPI-(DNA)-fluorescence on the other hand. In layer 4 of sensory cortices we find that neural somata and nuclei are of small and relatively homogeneous size. Nuclei of neural and non-neural layer 4 cells identified by NeuN-immunofluorescence had the same integrated DAPI-fluorescence. We also counted the number of chromocenters, bright spots of heterochromatic DNA fluorescence, the number of which often correlates with chromosome number. Layer 4 neurons and non-neural cells both showed a
similar number of ~19 chromocenters. These observations are consistent with a diploidy of layer 4 neurons. In layer 5 neurons, however, we observed much larger neurons and a remarkable size diversity of somata and nuclei. In primary visual cortex the integrated DAPI-fluorescence of layer 5 neurons was variable and showed multiple peaks. When normalized to non-neural cells, the largest peak in the integrated layer 5 neuron fluorescence distribution was found at 1.45-fold the average non-neural cell fluorescence. A smaller peak was found at 1.05-fold the fluorescence of non-neural cells. Chromocenter counts of visual cortex layer 5 neurons showed a wide distribution with a major peak at 26 to 27 chromocenters and a second smaller peak at 20 chromocenters. The variation in both DNA-fluorescence and chromocenter number, the distribution of multiple peaks at close to half-integer values is suggestive of polyploidy. Specifically, it may suggest that a majority of visual cortex layer 5 neurons is triploid and that another smaller fraction is diploid. Furthermore, in an even smaller (≤ 10%) fraction of layer 5 neurons with very large somata we observed fluorescence values and chromocenter counts suggestive of tetraploidy. There are clear areal size differences within cortical layer 5 and visual cortical neurons are on average smaller than neurons in auditory or somatosensory cortices. Auditory cortex layer 5 neurons showed significantly higher normalized DAPI-fluorescence and chromocenter counts than visual cortex neurons. Our auditory cortex layer 5 data are compatible with presence of a large fraction of tetraploid neurons and also suggest the presence of pentaploid neurons. Our evidence supports and extends earlier conclusions from flow-cytometry and spectroscopy, which also indicated the presence of tetraploid layer 5 neurons. We suggest that patterns of polyploidy shape the laminar and areal cell diversity of the cerebral cortex.

Disclosures: M. Brecht: None. J. Sigl-Gloeckner: None.

Poster
510. Striate Cortex Circuitry

Location: Hall A
Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 510.14/O2

Topic: D.04. Vision

Support: NSERC

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Université de Sherbrooke
Title: The 50-ms window of opportunity in V1 microcircuits

Authors: *V. BHARMAURIA¹, L. BACHATENE¹, S. CATTAN¹, N. CHANAURIA¹, J. ROUAT², S. MOLOTCHNIKOFF³;
¹Dept. Sci. Biologiques, Univ. De Montreal, Montreal, QC, Canada; ²Univ. de Sherbrooke, Sherbrooke, QC, Canada

Abstract: Neuronal subnetworks are critical in encoding sensory information reaching the brain; however, the knowledge of the properties of these feature-encoding networks is still sparse. Here we show through simultaneous recordings of neurons in the primary visual cortex of anesthetized cats that a cell-assembly frames a salient functional network (revealed by the cross-correlograms) in relation to a particular stimulus. The dynamics of this network-selectivity is characterized by change of functional connections from one orientation to another, wherein some connections remain stable, others disappear with the changing stimulus and new ones appear too. The peak probabilities in the cross-correlograms (that are an indication of weight of connections) also change as the orientation changes. The connected neurons within these networks cross-interact synergistically (an increase of ~50% of the firing rate) with each other in a characteristic 50-ms window (in relation to the spike of a reference neuron) that we call the temporal window of opportunity. Moreover, we found that there is an augmented gamma activity (30-80 Hz) for about 50 ms between the functionally connected neurons than the unconnected neurons in these circuits as a consequence of higher coherence in the gamma band between the connected neurons. Lastly, the higher gamma oscillations (60-80 Hz) are exclusively linked to the fast-spiking neurons. These results led us to postulate that in primary sensory areas information is processed within 50 ms after a salient functional network is formed within a cell-assembly. Furthermore this particular network exhibits stimulus selectivity.


Poster

510. Striate Cortex Circuitry

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 510.15/O3

Topic: D.04. Vision

Support: ERC starting grant NEUROOPTOGEN

A postdoctoral fellowship from the Alexander von Humboldt Foundation
Gain modulation by serotonin in the macaque primary visual cortex

**Authors:** *L. HRUBA*¹, T. OTT², A. NIEDER², P. POURRIAHI¹, H. NIENBORG¹; ¹Univ. Tuebingen, Ctr. For Integrative Neurosci. (AG Nienborg), Tuebingen, Germany; ²Univ. of Tuebingen, Inst. of Neurobio., Tuebingen, Germany

**Abstract:** The serotoninergic neuromodulatory system plays an important physiological role and has been implicated in a number of pathological conditions. However, despite anatomical evidence for substantial serotoninergic projections to the primary visual cortex (V1) and high expression of serotonin receptors in this brain area, little is known about how serotonin modulates visual processing in V1. Here, we used extracellular recordings in macaque V1 to examine the effect of serotonin on visual tuning properties. A macaque monkey was required to perform a standard fixation task (fixation for 2 sec within a 1 degree fixation window) while we presented sinusoidal grating stimuli inside the receptive field of single units in V1. We measured the effects of serotonin on tuning properties (binocular and monocular in each eye) for drifting gratings of varying orientation, spatial frequency, contrast and size. We recorded single units responses in V1 area while iontophoretically applying serotonin hydrochloride (10mM; pH=3.5; n=44 single units) or 0.9 % NaCl (pH=3.5; n=10 single units) as a control. The range of ejection currents was between 2 to 50nA (median: 10nA) for iontophoresis with serotonin and 10 to 50nA (median: 20nA) for the control iontophoresis using NaCl. We found that serotonin mainly decreased visual responses to a given stimulus while typically no changes were found after NaCl application. Across the neuronal population the changes induced by serotonin were mainly explained by a decrease in neuronal response gain, rather than additive changes. On average the gain was reduced to 72% of the gain without serotonin application, significantly different (p<0.005) from the results for iontophoretic NaCl application, while we found no significant additive change across the population. The sizes of the effects for the dominant and non-dominant eye were highly correlated (r = 0.68; p<0.0001 for multiplicative changes and r = 0.66; p<0.0001 for additive changes). In summary, our data showed that across a variety of stimulus dimensions serotonin predominantly decreased visual responses in macaque V1 by multiplicative, not additive changes.

**Disclosures:** *L. Hruba: None. T. Ott: None. A. Nieder: None. P. Pourriahi: None. H. Nienborg: None.*

**Poster**

*511. Mapping Connectivity and Function of Extrastriate Cortex*

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#: 511.01/O4**
Topographic organization of the “third tier” dorsomedial visual cortex in the macaque monkey

Abstract: The extent and topographic organization of visual areas located anterior to V2 in macaques remains a matter of some contention. One of the most controversial aspects is the layout of areas on the dorsomedial aspect of the occipital lobe, in the depths of the lunate and parietooccipital sulci, and annectant gyrus. According to the most commonly accepted interpretation, this region includes dorsal area V3 (V3d), laterally, and another area (referred to as either V6 or PO, according to different definitions), medially. Surprisingly, no electrophysiological study to date has included a comprehensive exploration of this region. Thus, the nature of the topographic transition that marks the putative border between V3d and V6/PO has remained unresolved. We explored the dorsomedial cortex in 3 macaques in acute recording sessions under sufentanil/N2O anaesthesia. The brains were sectioned in the parasagittal plane, allowing the precise reconstruction of >1,000 recording sites relative to myeloarchitectural boundaries. The data were reconstructed in bi-dimensional maps using the software CARET. Rostral to the anterior border of V2 (marked by a representation of the horizontal meridian), we found a representation of the lower quadrant, which had a continuous representation of the inferior vertical meridian at its rostral border. This region formed a smooth eccentricity gradient, from central (laterally, in the annectant gyrus) to far peripheral (>50°, in the parietooccipital sulcus). There was no topographic transition at or near the border between putative areas V3d and V6/PO. Rather, the lower quadrant representations that are usually interpreted as belonging to these areas merged into each other, forming a single map. Moreover, near the midline this map
continued into a representation of the upper peripheral visual field. Receptive fields in the upper central visual field were found near the lateral limit of our recording area, in the annectant gyrus, near the putative V3 border, but additional experiments will be needed to clarify the visual topography in this region. Our findings show similarities with evidence obtained in New World marmoset monkeys, and point towards a reinterpretation of the visuotopic organization of the cortex lying rostral to macaque V2.


Poster

511. Mapping Connectivity and Function of Extrastriate Cortex

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 511.02/O5

Topic: D.04. Vision

Support: Intramural Research Program NIH

Title: Evaluating the correspondence between category-selective and retinotopic organization of the lateral human occipitotemporal cortex

Authors: *E. H. SILSON1*, I. I. A. GROEN1, D. J. KRAVITZ2, C. I. BAKER1;
1Lab-Brain & Cog, Natl. Inst. of Mental Hlth., Bethesda, MD; 2Dept. of Psychology, The George Washington Univ., Washington, DC

Abstract: The organization of lateral human occipitotemporal cortex (OTC) has been characterized according to two main principles: functional specialization (category-selectivity) and maps of the visual field (retinotopy). Recently, converging evidence from functional imaging and neurostimulation studies highlights that these organizing principles are not mutually exclusive, and indeed can be present in the same location. Here, we combined detailed mapping of both population receptive fields and category-selectivity to examine this spatial relationship within lateral occipitotemporal cortex across a large number of subjects (n=16). We focus in particular on a region of OTC defined as scene-selective (scenes > objects or faces), often referred to as the Transverse Occipital Suleus (TOS). TOS covers a large swath of cortex, and we have previously shown that it has an overall bias for the contralateral lower visual field. To compare category-selectivity and retinotopy, we first delineated visual field maps (V1-V3d, V3A, V3B, LO1, LO2 & V7) on the lateral surface based on their representations of the visual field in all 32 hemispheres. Second, we calculated the proportion of spatial overlap between TOS
and these maps. Across both hemispheres, TOS exhibited maximal overlap with visual field maps V3B and LO2 - a pattern that increased when the threshold for category-selectivity was increased. There was a vanishingly small overlap with early visual areas V1-V3d and more modest overlap with V3A, LO1 and V7. Third, we assessed the frequency with which the peak of scene-selectivity fell within the boundaries of these maps. Across all hemispheres, the peak of scene-selectivity was located within the boundaries of either V3B or LO2 to a higher frequency than the other field maps or non-retinotopic cortex. The fact that TOS overlaps predominately V3B and LO2 is intriguing. Our measurements of visual field coverage within these maps indicate that whereas V3B represents the entire contralateral hemifield, LO2 represents predominantly the contralateral lower quadrant. The differing representations within V3B and LO2 may reflect differential sensitivity to particular visual features present within scenes. More generally, the combined lower field representations within LO2 and V3B likely account for our previous measurement of an overall contralateral lower field bias in TOS. Evaluating the relationship between category-selectivity and retinotopy is crucially important for neurostimulation studies of category-selectivity, which invariably target a single focal location. Overall, there appears no simple relationship between TOS and a single map of the visual field.


Poster

511. Mapping Connectivity and Function of Extrastriate Cortex

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 511.03/O6

Topic: D.04. Vision

Support: NIMH DIRP

Title: Mapping spatial patterns of whole brain MRI using simultaneously recorded single neurons

Section on Cognitive Neurophysiol. and Imaging Lab. of Neuropsychology, NIMH/NIH, Bethesda, MD

Abstract: Neurons interact with each other and integrate information over many spatial scales. At large scales, functional magnetic resonance imaging (fMRI) during rest reveals correlational maps thought to reflect specialized brain networks. At finer scales, hundreds of thousands of
adjacent neurons within each voxel of gray matter fire action potentials asynchronously. Linking these scales, the timing of a spike is often determined by specific afferent input to a neuron from another brain region. Here we report functional interactions between individual neurons and fMRI signal fluctuations recorded simultaneously throughout the brain. We recorded spontaneous spiking activity of single units in the anterior fundus face patch of the superior temporal sulcus (STS) of awake monkeys during whole-brain fMRI. A chronically implanted microwire bundle allowed for concurrent recordings from many neurons including sequential recordings from the same neurons isolated across multiple weeks. Functional maps were computed based on correlations between the spike trains of each cell and fluctuations in hemodynamic signals throughout the brain. These revealed discrete areas of robust correlation with changes in spike rate accounting for up to one third of the fMRI signal variance in some voxels. Maps from single neurons were reproducible within recording sessions and across recording sessions spanning several weeks, although the magnitude and borders of these maps varied with shifts in arousal (eyes open vs. eyes closed). Across the population, neurons measured from within an estimated radius of ~500 µm (much smaller than one voxel) gave rise to a striking diversity of maps. The majority of neurons showed correlations with fMRI signal within the STS; these were widespread for some cells and highly localized for others. Individual cells likewise varied in the extent to which their activity was correlated with fMRI signal in occipital visual cortex. Some neurons also showed correlations with hemodynamic signals in distinct areas such as the frontal and cingulate cortices. These results reveal a rich diversity of neural activity from adjacent neurons within a single voxel and give new insight into the functional interactions of individual cells with other brain regions.


Poster

511. Mapping Connectivity and Function of Extrastriate Cortex

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#Poster#: 511.04/O7

Topic: D.04. Vision

Support: NIH DP1 EY023176

Title: Dissecting the wiring diagram and function of cortico-cortical feedback from LM to V1 in mice
Authors: *S. SHEN, X. JIANG, J. REIMER, A. TOLIAS;
Neurosci., Baylor Col. of Med., HOUSTON, TX

Abstract: The mammalian visual system is composed of hierarchically organized cortical areas, which are reciprocally connected with feed-forward and feedback projections. Compared to feed-forward pathways, much less is known about the connectivity and function of feedback pathways. In mice, we found that the lateral medial area (LM) sends extensive feedback projections to the retinotopically corresponding area in primary visual cortex (V1). To characterize the wiring diagram of these feedback projections, we expressed channelrhodopsin2 (ChR2) in LM pyramidal cells, and performed multiple simultaneous whole-cell recordings of pyramidal cells, parvalbumin (PV), somatostatin (SST), and vasoactive intestinal peptide (VIP) expressing cells in L2/3, L4 and L5 of V1, while photostimulating feedback axon terminals. Although all these cell types exhibited monosynaptic excitatory responses to feedback activation, the amplitudes of their responses varied greatly depending on the cell types and layers. We found that among all the cell types, L2/3 PV and L2/3 SST had the largest response amplitude of EPSC and EPSP, 2-4 times as large as those of L2/3 pyramidal cells recorded in the same slice. To further reveal the net effect of feedback excitation to V1 pyramidal cells, we drove V1 pyramidal cells to fire by either injecting depolarizing currents in *in vitro* slice recordings, or presenting grating stimuli to the animal in *in vivo* whole-cell recordings, and on top of those, photostimulated the feedback projections. We found that both *in vitro* and *in vivo*, optogenetic excitation of feedback elicited a short depolarization followed by a long hyperpolarization of V1 pyramidal cells, creating a narrow temporal window of excitation that allows pyramidal cells to fire in a precise time. Our results suggest that feedback projections from LM to V1 help precisely time the firing of V1 pyramidal cells, thus potentially modulating their temporal coding.

Disclosures: S. Shen: None. X. Jiang: None. J. Reimer: None. A. Tolias: None.

Poster

511. Mapping Connectivity and Function of Extrastriate Cortex

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 511.05/O8

Topic: D.04. Vision

Support: NIH 1R01EY02391501A1

Title: Cortex based alignment improves the intersubject alignment of cytoarchitectonic regions in the human ventral stream
Authors: *M. ROSENKE*, K. S. WEINER, M. FROST, M. BARNETT, K. ZILLES, K. AMUNTS, R. GOEBEL, K. GRILL-SPECTOR;


Abstract: Over the last 15 years, the Jülich group has developed observer independent methods to delineate cytoarchitectonic regions in the human brain (Amunts et al. 2000). These methods have identified 8 cytoarchitectonic regions in the human ventral stream. An initial four regions span the occipital lobe, ascending from hOc1 to hOc4v. An additional four regions continue to the temporal lobe and tile the fusiform gyrus (FG) ascending from FG1 to FG4 (Caspers et al. 2013; Weiner et al. 2014; Lorenz et al. 2015). While there is general consensus that hOc1 corresponds to striate cortex and is anatomically consistent across subjects (Fischl et al. 2008), there is little agreement that such a correspondence between cytoarchitectonic regions (cROIs) and cortical folding patterns persists in higher-order regions. We hypothesized that if such a correspondence exists, cortex based alignment (CBA) of cROIs will yield better registration across subjects than the standard volume based alignment to the MNI template. To test this hypothesis, we created cortical surface reconstruction of 10 postmortem brains in which these 8 cROIs were identified. We then used BrainVoyager to compare the registration of cROIs across subjects using three methods: (1) volume-based alignment to the MNI template, (2) CBA to the Freesurfer average brain (based on 39 independent brains, CBAfs), and (3) CBA to the average postmortem brain using the MFS as an additional constraint (CBApm+MFS). Our results show that both CBAfs and CBApm+MFS significantly improve the across subject correspondence of extrastriate cROIs compared to volume based alignment (F(2,14) = 153.9, p < .001, mean proportion overlap of cROIs: volume-based: 0.19±0.03, CBAfs: 0.29±0.04, CBApm+MFS: 0.30±0.04). Compared to CBAfs, CBApm+MFS does not additionally improve the overlap of cROIs across subjects (t(15) = 1.7, p = .1), even for FG1-FG4 (t(7) = 1.2, p = .25, CBAfs: 0.31±0.06, CBApm+MFS: 0.32±0.05). However, compared to CBAfs, CBApm+MFS significantly reduces intersubject variability in the location of the FG1/FG2 boundary as well as the FG3/FG4 boundary (t(127) = 3.2, p = .002, mean pairwise distance: CBAfs: 3.09±2.33 mm, CBApm+MFS: 2.46±1.69mm). Overall, these results suggest that CBA of cytoarchitectonic regions enables more accurate atlases of the cytoarchitectonic segregation of the ventral stream by preserving the location of cROIs relative to cortical folding patterns, and by improving between-subject alignment. Furthermore, adding the MFS as an additional constraint significantly improves the between-subject convergence of cytoarchitectonic boundaries between FG1 and FG2, as well as FG3 and FG4.
Poster

511. Mapping Connectivity and Function of Extrastriate Cortex

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 511.06/O9

Topic: D.04. Vision

Support: Paul G. Allen and Jody Allen

Title: GCaMP6 fluorescence-based retinotopic mapping reveals medial areas and complementary representations in the mouse visual cortex

Authors: *J. ZHUANG¹, D. WILLIAMS², M. VALLEY¹, M. GARRETT¹, J. WATERS¹;
¹Neural Coding Dept., ²Allen Inst. for Brain Sci., Seattle, WA

Abstract: The mouse visual cortex includes multiple distinct functional areas, each with its own representation of visual space. Here we locate 3 previously unidentified visual areas in retinotopic maps from awake mice, generated by widefield imaging of GCaMP6 fluorescence. A 50 ms visual stimulus evoked changes in GCaMP6 fluorescence that could be >2 orders of magnitude larger and 5 times faster than changes in tissue autofluorescence. The large signal and fast kinetics of widefield GCaMP6 imaging enabled rapid mapping: 15 minutes of imaging was sufficient to identify most known visual areas. More prolonged imaging and increased averaging revealed retinotopic organization that extended into retrosplenial and primary somatosensory cortices and included two medial areas (MMA and MMP) and one anterior area (RLL), the latter entirely within barrel cortex. The four positive field sign areas bordering V1 (LM, RL, PM, P) exhibit complementary coverage, comparable to V1, with modest duplication of the central hemifield. The remaining areas each represented smaller regions of visual space, mostly near the center of the hemifield. Our results extend our understanding of the organization of mouse visual cortex to include up to 15 retinotopically distinct areas, some with complementary representations of visual space.

**511. Mapping Connectivity and Function of Extrastriate Cortex**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 511.07/O10

**Topic:** D.04. Vision

**Support:** Division of Intramural Research Programs, National Institute of Mental Health, National Institutes of Health.

National Institutes of Health; Grant number: MH70941; Grant sponsor: McDonnell Center for Higher Brain Function

**Title:** 3D interactive anatomical connectivity atlas of the claustrum in the macaque monkey

**Authors:** *K. S. SALEEM¹, D. GLEN², Z. SAAD², M. MISHKIN¹, J. L. PRICE¹;
¹Lab. Neuropsychology (LN), ²Scientific and Statistical Computing Core, Natl. Inst. of Mental Hlth. (NIMH/NIH), Bethesda, MD; ³Anat. and Neurobio., Washington Univ. Sch. of Med., Saint Louis, MO

**Abstract:** Until recently, researchers have relied largely on printed articles and atlases for connectivity information and anatomical references. While useful, this traditional format can be difficult to combine with imaging studies where the interconnections of numerous areas need to be understood simultaneously. Here we present a new software interface to show the extrinsic connections of the claustrum with different cortical areas in the macaque monkey using anatomical tracing methods. Restricted injections of retrograde and bidirectional tracers were made in subregions of the prefrontal (orbital, medial, lateral), temporal (superior, inferior, medial), inferior parietal, cingulate and retrosplenial cortices. The distribution of retrogradely labeled cells in the claustrum that project to these cortical areas was plotted in the histology sections using a microscope digitizer system. The main findings are: The labeled cells were found throughout most of the rostrocaudal extent of the claustrum after orbital, medial, and lateral prefrontal injections (e.g., areas 13m/l, 10mr, and 9d/46d/45a, respectively). A similar distribution of labeling was observed after restricted tracer injections into the caudal inferior parietal (area 7a), posterior cingulate (areas 23b and v23b), and the rostral belt and parabelt regions of the auditory cortex (RTL/RPB) in the superior temporal gyrus. In contrast, cell labeling was restricted mainly to the mid-portion of the claustrum after the inferior temporal lobe (areas TEad, TEav, and STSv) injections, or to both the mid- and rostral-portion of the claustrum after the rostral superior temporal gyrus (STGr) injections. Injections into the medial temporal cortex revealed a much stronger projection from claustrum to parahippocampal cortex (areas TF/TH) than to perirhinal cortex (areas 35/36). The dorso-ventral distribution of labeling varied between different injection cases but was restricted mainly to the ventral two-thirds of the
These anatomical data are also implemented in software interface AFNI (Analysis of Functional NeuroImages; Cox 1996) and SUMA (Surface Mapper; Saad and Reynolds 2012) allowing the users to navigate the connectional data interactively in 3D, and integrate the information directly with their imaging results in volumetric and surface modes. The connectional data are in register with the Saleem and Logothetis MRI anatomical template atlas, and corresponding segmentation and cortical surfaces (Reveley et al, in preparation). Users can also display the cortico-cortical connections of individual cortical areas within this software interface (Saleem et al, OHBM 2015, Abstr 5109).


Poster
511. Mapping Connectivity and Function of Extrastriate Cortex

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 511.08/O11

Topic: D.04. Vision

Support: NIMH DIRP

Title: Comparing fMRI maps derived from seed voxels, local field potential, and spiking activity during rest

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Abstract: Functional magnetic resonance imaging (fMRI) of spontaneous brain activity reveals spatial patterns of synchronization thought to reflect interactions among regions making up distinct functional networks. One way to study these interactions is to measure the temporal correlations of the whole brain with a localized seed voxel or fMRI region of interest. Within a single voxel, however, there is a rich diversity of neural signals showing many patterns of activity. Here we investigate how fMRI maps vary when they are derived from different types of neural signals measured simultaneously from within a voxel using a seed-based approach. We recorded spontaneous local field potential (LFP), multunit activity (MUA), and single unit responses from MR-compatible microwire bundles located within a voxel in the macaque superior temporal sulcus. These neural signals were recorded simultaneously with regional
cerebral blood volume changes during whole-brain fMRI acquisition. We obtained functional maps by correlating the time courses of either LFP power or spiking activity with hemodynamic signal fluctuations in each voxel. We then compared these maps to those obtained using an fMRI-based seed voxel located at the electrode tip. Functional activity maps obtained from the LFP power depended upon frequency content, and resembled results obtained previously from other brain areas (Schölvinck et al, (2010) PNAS). Maps derived from the gamma and theta LFP ranges showed a broad positive correlation throughout the cerebral cortex and generally resembled fMRI seed maps from voxels near the electrode tip. By contrast, maps obtained from the beta range recorded from the same electrodes showed more localized correlations that were more variable across sessions. Functional maps derived from spiking activity differed among neurons. MUA maps obtained from some channels were similarly broad, whereas others were more spatially restricted in their functional correlations. Maps based on isolated single units were generally more localized and dissimilar to one another than those derived from either MUA or LFP signals. Thus, the various types of spontaneous neural signals that can be measured and tracked within a single voxel at a given cortical position give rise to diverse but reliable whole-brain correlational maps using this method. We will address how particular signal-specific maps may provide insight into different components of resting-state brain activity.


**Poster**

511. Mapping Connectivity and Function of Extrastriate Cortex

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 511.09/O12

**Topic:** D.04. Vision

**Support:** NIH grant MH 93567

**Title:** Calcium-binding proteins immunoreactivity in macaque V2 indicates differential population expression across cortical regions

**Authors:** *J. J. COPPOLA, A. A. DISNEY; Vanderbuit Univ., Nashville, TN

**Abstract:** Inhibitory interneurons of the primate cortex comprise heterogenous populations, with considerable structural and functional diversity. Traditionally, these populations were classified based on their morphologies. More recently, immunocytochemical and genetic markers have
become a prevalent alternative for classification, with well-documented qualitative and quantitative data. In the present study, we use immunocytochemical markers to quantify the distribution and overlap of three neuronal classes in visual area V2. Expression of the calcium-binding proteins calbindin (CB), calretinin (CR) and parvalbumin (PV) by inhibitory interneurons has been found to define almost entirely non-overlapping classes of cells that, together, are believed to account for approximately 95% of the inhibitory neurons in the primary visual cortex (V1) of the macaque. Similarly, these proteins have been shown to represent distinct populations of neurons in the monkey prefrontal cortex (PFC). In the present study, triple immunofluorescence labeling was used to identify neurons expressing CB, CR, and PV in V2 of three macaques. Laminar distribution and degree of co-labeling for each population were quantified. 1146 calcium-binding protein-immunoreactive neurons were counted in a total area of approximately 7.587mm². Our results indicate that these calcium-binding proteins represent non-overlapping populations in macaque V2; no neurons were found to be triple labeled, or to be dual labeled for CR/CB. Less than 1% of all cells were dual labeled for CB/PV (0.785%), and less than 1% were dual labeled for CR/PV (0.175%). Overall, we found PV-immunoreactive neurons to account for 52% of the calcium-binding protein-immunoreactive population, with CB-immunoreactive neurons accounting for 25%, and CR-immunoreactive neurons accounting for 23% of the same population. Previously in macaque V1, a significant majority of inhibitory neurons was found to be PV-immunoreactive. In the macaque PFC, however, these proportions shift such that CR-immunoreactive neurons are the largest population. Our data indicate that the expression of these three populations of calcium-binding protein-immunoreactive neurons in V2 is distinct from that found in V1 and in PFC, and support the use of CB, CR, and PV as immunocytochemical markers for non-overlapping neuronal classes.

Disclosures: J.J. Coppola: None. A.A. Disney: None.

Poster

511. Mapping Connectivity and Function of Extrastriate Cortex

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 511.10/O13

Topic: D.04. Vision

Title: Effects of TBS on visual cortex excitability depend on intensity as well as on coil geometry

Authors: S. BRÜCKNER, *T. KAMMER;
Univ. of Ulm, Ulm, Germany
**Abstract:** Transcranial magnetic theta burst stimulation (TBS) is an efficient method to modulate excitability of the human motor cortex, but it is still not clear whether the scheme, i.e., inhibitory effects by continuous TBS (cTBS) and excitatory effects by intermittent TBS (iTBS) can be transferred to other cortical regions. In 4 independent experiments we investigated the effect on the excitability of the visual cortex, using the individual phosphene threshold (PT) as dependent variable. In experiment 1 (n=20) and 2 (n=20), we applied either cTBS or iTBS with 100% PT intensity using a figure-of-eight-coil, respectively. In experiment 3 (n=15) and 4 (n=15), cTBS was applied with 80% PT intensity using either a figure-of-eight-coil (exp.3) or a round coil (exp.4). PT was measured before and 2min after TBS. Applying TBS with 100% of individual PT intensity on visual cortex we observed a significant modulation of PT with neither cTBS nor iTBS. However, cTBS decreased PTs numerically. As Franca et al. (2006) showed an inhibitory effect of cTBS on phosphene thresholds using 80% PT intensity and a round coil, we aimed to vary cTBS intensity and coil type. In experiment 3, we therefore applied 80% PT intensity with the figure-of-eight-coil used in experiments 1 and 2. A paired t-test showed decreased PTs following cTBS (t=2.4; p= 0.031), indicating a facilitatory effect. Using the round coil in experiment 4, no such effect was found (t=0.91, p=0.38). iTBS applied to the visual system seems not to modulate excitability. Our negative results using 100% of PT are in line with the non-finding of Franca et al. (2006) using 80% PT intensity. For cTBS, subthreshold intensities (80% PT) seem to be most effective in modulation of PT. An increase in intensity (100% PT) does not increase the modulatory effect, suggesting a non-linear system behavior. Surprisingly, in our data cTBS effects on visual cortex depend on coil geometry. Applying cTBS with 80% PT intensity using a figure-of-eight-coil had a facilitatory effect, whereas the effect using a round coil pointed in the opposite direction. This tendency to inhibition is in line with the effects reported by Franca et al. (2006). Thus cTBS effects in the visual system might depend on the focality of the applied electromagnetic field varied by the different coil geometries. The observed facilitatory effects applying cTBS with the figure-of-eight coil is in contrast to the canonical description in the motor system (Huang et al. 2005). Although we and others observed significant mean group effects in some protocols of TBS, with other protocols no effects were obtained. This points to the fact that TBS effects might act in different directions across individuals.

**Disclosures:** S. Brückner: None. T. Kammer: None.

**Poster**

511. Mapping Connectivity and Function of Extrastriate Cortex

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 511.11/O14
Abstract: Neurons with similar response preferences are found clustered throughout macaque ventral visual pathway, with fMRI-identified patches showing largely homogeneous categorical responses to stimuli. Recently we reported that, while single neurons in the anterior fundus (AF) face patch responded consistently to multiple viewings of a natural video, their response time courses were often only weakly correlated across nearby neurons (< 500 µm) (McMahon et al., 2015). Here, we asked whether this diversity could be investigated by harnessing the spatial coverage of fMRI to map aspects of the functional information carried by individual neurons at the level of the whole brain. We therefore developed an approach that applied a combination of fMRI and single unit recordings to create a whole-brain fMRI readout based on individual neural responses. Subjects freely viewed a number of 5-minute movies replete with macaque and human social interaction (Russ et al., 2015). In some sessions, single unit responses were collected from the AF face patch, in others fMRI responses were recorded over the whole brain, and in some sessions the two measurements were conducted simultaneously. Each video stimulus served as a common currency for driving brain activity, which thus allowed us to assess the relationship between single unit and fMRI responses. Whole-brain functional maps were computed for each neuron as a matrix of correlation coefficients, with each voxel’s value being the correlation between its fMRI time course the AF neuron’s activity. This approach revealed that neurons located within a few hundred microns produced maps consisting of distinct, and often very different, combinations of visual areas. In general, individual AF neurons produced maps that featured positive or negative correlations with known face patches along with other subregions of the superior temporal sulcus. Many neurons, however, also showed positive or negative correlations with other areas, including the retinotopic visual cortex and prefrontal regions. As a population measure, we produced maps based on time courses of a set of principal components, or “eigenneurons,” derived from the AF single unit responses. Results of this analysis revealed distinct spatial patterns of shared responses within the AF population. Finally, mapping the gamma power fluctuations of the AF local field potential revealed positive correlations in many of the specific regions measured from single units. This approach provides
a new means to investigate the diverse signals embedded within a local population of neurons by revealing the whole-brain functional pattern correlated with individual neurons.


Poster
511. Mapping Connectivity and Function of Extrastriate Cortex

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 511.12/O15

Topic: D.04. Vision

Support: RO1EY02391501A1

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NSF DGE-114747

Title: Macromolecular tissue properties of human high-level visual cortex develop with age and may shape cortical function

Authors: *J. GOMEZ*1, M. BARNETT1, V. NATU1, A. MEZER2, K. GRILL-SPECTOR1;

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Abstract: Computations performed by a given cortical region are constrained by its underlying cytoarchitecture and likely sculpted through development. Novel quantitative magnetic resonance imaging (qMRI) methods (Mezer 2013) have enabled unbiased *in vivo* measurements of macromolecular tissue properties, revealing development of macromolecular tissue volume and relaxivity in human white matter (Yeatman 2014). In parallel, other research has illustrated functional development in human ventral temporal cortex (VTC, Golarai 2007). However, it is unknown if there are concurrent changes in gray matter tissue and if they are related to the development of function. Here, we investigated how cortical tissue properties measured via qMRI develop with age from childhood (n=23, 5-12 years) to adulthood (n=20, 22-25 years) and how this measure of gray matter relates to functional selectivity. To measure cortical development consistently across subjects, we focus on anatomical ROIs of lateral and medial VTC. All subjects completed a multi-flip qMRI scan (protocol of Mezer 2013). We quantify in each subject both macromolecular tissue volume fraction (MTV) and spin-lattice relaxation rate (T1) per voxel. To test the relation between the development of anatomical properties to functional properties, each subject also underwent an fMRI localizer experiment to identify face-
selective regions on the lateral fusiform gyrus (pFus- and mFus-faces) and medial place-selective regions on the collateral sulcus (CoS-places/PPA). In both lateral and medial anatomical partitions of VTC, we observe a developmental shift in the voxelwise distribution of T1 and MTV values in gray matter. T1 values decrease from childhood to adulthood (mean decrease of 4.8%; KS-test Ps<10-50) and MTV values increase from childhood to adulthood (mean increase of 7.2%; KS-test Ps<10-50). Interestingly, in adults and children, the distributions of T1 values in pFus/mFus-faces differ from CoS-places (Ps< 10-14), suggesting that different functional regions have different gray matter properties. Additionally, this relationship varies across development. From childhood to adulthood, we observe a fivefold increase in the separability (d') of voxelwise T1 distributions between pFus/mFus-faces and CoS-places. Our findings suggest a novel structure-function relationship in human VTC, where anatomical development of the local tissue may contribute to shaping its cortical function.


Poster

511. Mapping Connectivity and Function of Extrastriate Cortex

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 511.13/O16

Topic: D.04. Vision

Title: V1 population activity can drive development of highly diverse receptive fields in extrastriate cortex

Authors: *B. W. MEL1, R. JAIN2;

Abstract: The mechanisms underlying the development of area V1 have been extensively studied both experimentally and with computer models. V1 is unusual among visual cortical areas, however, in that the majority of V1 cells respond well to the same basic shape -- an elongated edge or grating-like feature with varying preferences for orientation, spatial frequency, and other parameters. Reflecting this homogeneity, the core of most V1 models consists of a set of Gabor filters with varying orientation and scale parameters densely tiling the visual field. In contrast, mid-level visual areas such as V2 and V4 contain neurons selective for a multitude of diverse shape and/or texture features (Anzai, Peng, & Essen, 2007; Gallant, Braun, & Van Essen, 1993; Movshon & Simoncelli 2015; Pasupathy & Connor, 1999, 2001), but little is known as to what drives the development of such diverse RF properties in these areas. Given that V1 is a
major source of input to mid-level visual areas, we hypothesized that natural images that strongly activate local populations of V1 cells could provide the rich input statistics needed to drive the diversification process in extrastriate areas. To explore this, we collected natural image patches that strongly activated neighborhoods of cells in a V1 model, while varying (1) the size of the V1 neighborhood over which responses were pooled, and (2) the diversity of RF profiles within the V1 neighborhood, reflecting recent evidence that V1 profiles themselves have significant natural shape variation (e.g. Cossell et al. 2015). These image patches were then used to drive an unsupervised learning process in a model extrastriate area (hereafter EA), and the resulting RF structures were analyzed. We found that increasing the size of the V1 pooling neighborhood increased RF diversity in EA, but promoted the formation of RFs sensitive to repeating textures (i.e. "stuff" -- See Movshon and Simoncelli, 2015). In contrast, increasing the diversity of V1 RF profiles, to include curved Gabor-like profiles in addition to the conventional straight ones, led to an explosion of RF types more attuned to object boundary and junction structures (i.e. "things"). These finding provide a novel account for the formation of different types of RF diversity in mid-level visual cortex.

Disclosures: B.W. Mel: None. R. Jain: None.

Poster
511. Mapping Connectivity and Function of Extrastriate Cortex

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 511.14/O17

Topic: D.04. Vision

Support: Fondecyt 1120124

Title: Visual cortex in birds? Anatomical and physiological evidence favors a cortical organization for the intrinsic neural circuitry of the avian visual pallium

Authors: M. FERNANDEZ1, P. AHUMADA1, C. NORAMBUENA1, *J.-C. LETELIER2, G. MARIN1,2, J. MPODOZIS1;
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Abstract: The mammalian cortex has two prominent features: lamination of characteristic neuronal types (including primary sensory neurons), and radially arranged projections between cortical layers, forming anatomical and physiological columnar modules. In contrast, the avian sensory pallium has long been considered as a necklace of sequentially related nuclei, lacking of
cortical features such as a columnar organization or recurrent interactions between their components. These seeming differences have been questioned by several recent studies addressing the organization of the neural circuits embodied in the sensory areas of the avian pallium. Recent studies from our Laboratory have shown that the visual sensory pallium is composed of three differentiated, layers: internal (Entopallium, E, thalamo-recipient), intermediate (intermediate nidopallium, NI, efferent) and external (ventral mesopallium, MV, associative), highly interconnected between them by a system of dorso-ventrally oriented, discrete and homotopic columnar bundles of axons. The detailed morphology of the cells and processes composing the columns, as well as the physiological operation of them, has not been investigated in detail. We have addressed these issues practicing intracellular cell fillings in chick brain slices, and current source density analysis (CSD) in anesthetized pigeons. Our results confirm that the neuronal axonal processes connecting these three layers are mostly dorso-ventrally oriented. Characteristic “column-forming” cell types of each layer were identified, including cells in the E and NI projecting to the basal ganglia. CSD maps elicited by the electric stimulation of discrete E loci show a layered/sequential arrangement of synaptic activation, with characteristic sinks in each layer. Finally, local electric E stimulation elicited at the MV spike activity restricted to the columnarily homotopic locus. Previous studies have reported that the E and the NI-MV share molecular identity with cortical layer 4 and layer 2/3 respectively. Taken together, these results strongly support a cortical-like organization for the avian visual pallium.


Poster

511. Mapping Connectivity and Function of Extrastriate Cortex

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 511.15/O18

Topic: D.04. Vision

Title: Mapping the visual system devoid of visual stimuli

Authors: *A. MENDELSOHN¹, S. GABAY²;
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Abstract: The visual cortex represents incoming information according to distinct organization principles, one of which is eccentricity, whereby the object’s distance from the center of gaze is represented along the posterior-to-anterior axis in V1. The ventral stream, known for its role in visual categorization and object recognition, obeys a related organization principle, such that
large entities are represented in medial portions, and smaller objects are represented more laterally. Despite the apparent link between the organizational principles in V1 and along the ventral stream, a direct link between these organization systems is lacking. Here we examined resting-state fMRI data using a novel functional connectivity technique that capitalizes on mapping brain areas according to functional correlations with distinct structural properties. Anatomical datasets of 31 participants from the NKI-Rockland Data program were segmented, and V1 voxels were delineated for each participant. Pearson correlations were then computed between fMRI time-courses of each cortical voxel and each and every voxel in V1. Finally, each cortical voxel was color-coded according to the y-coordinate (anterior-to-posterior) in V1 that had the maximal correlation with that particular voxel. Using this technique, a lateral-to-medial gradient was detected in ventral temporal cortex, whereby lateral-to-medial ventral temporal cortex was maximally correlated with posterior-to-anterior V1. In agreement with recent findings in the lateral surface of temporal cortex in response to objects with varying representational sizes, we detected a similar pattern, showing that ventro-lateral vs. dorso-lateral temporal lobes were maximally correlated with posterior vs. anterior V1, respectively. These results indicate that the retinotopic principle of visual eccentricity is detected in ventral and lateral temporal cortex, and importantly - in the absence of visual stimuli, revealing an anatomical infrastructure for the representation and categorization of visual information.

Disclosures:  A. Mendelsohn: None. S. Gabay: None.

Poster

511. Mapping Connectivity and Function of Extrastriate Cortex

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

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Topic: D.04. Vision

Support: NIH Grant EY022090
Title: Distinct balance of excitatory and inhibitory drive within feedforward and feedback pathways in mouse visual cortex

Authors: *R. D’SOUZA*¹, Q. WANG², A. M. MEIER¹, A. BURKHALTER¹;

Abstract: Cortical areas communicate with each other via axonal projections that terminate in distinct laminar patterns that depend on whether the projections are feedforward (FF) or feedback (FB). Mouse visual cortex is composed of at least ten distinct areas that are interconnected by FF and FB pathways. To study the “interareal” communication between visual areas, we first determined the hierarchical positions of three such areas - V1, LM, and PM. We examined the laminar projection patterns of axons originating from each of these three areas to each of the other nine. FF axons originating in V1 tended to selectively target layers (L) 2-4 while avoiding L1, while FB projections to V1 preferentially terminated in L1. We therefore used the ratio of the optical density of projecting axons in L2-4 to that in L1, as a measure for quantifying the hierarchical level of each area. V1 had the highest L2-4:L1 projection ratio (and therefore the lowest position in the hierarchy), followed by LM, with PM holding the highest hierarchical position among these three areas. We next examined the cellular and synaptic properties of FF and FB pathways. The primary neuronal targets of interareal connections in the visual cortex are the pyramidal (Pyr) cells and the parvalbumin (PV)-expressing, GABAergic interneurons. Activated PV cells inhibit local Pyr cells; PV cells therefore provide feedforward inhibition (FFI) between visual areas. The strength of FFI within a circuit can determine the selectivity of signal transmission by restricting the time window for the integration of excitatory inputs. Therefore, to understand how visual signals are routed through the cortical hierarchy, we measured the strength of monosynaptic interareal inputs to Pyr and PV cells in FF and FB pathways. To do this we employed subcellular Channelrhodopsin (ChR2)-assisted circuit mapping in acute slices from 30-45 days old PV-Cre X Ai9 mice, in which PV cells express the fluorescent protein tdTomato. Excitation of ChR2-expressing axon terminals was achieved by a blue laser delivered in a grid pattern across all layers, and EPSCs were measured from neighboring Pyr and PV cells. Our results indicate that the interareal excitation of PV cells, relative to that of Pyr cells, is stronger in the FF than in the FB pathway, and stronger in L2/3 than in L5. This suggests more potent FFI in FF than in FB pathways in L2/3, with an overall lower level of FFI in L5. Further, synaptic inputs in L1 were stronger in FB than in FF pathways. We propose that FF circuits in L2/3 are selective for synchronous inputs, while FB projections more broadly connect to Pyr cells thus efficiently regulating their responses to FF input in a context-dependent manner.
Disclosures:  R. D'Souza: None.  Q. Wang: None.  A.M. Meier: None.  A. Burkhalter: None.

Poster

512. Sensorimotor Transformation: Higher Order Functional Organization

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 512.01/O20

Topic: D.05. Visual Sensory-motor Processing


Title: State-dependent processing in the brain

Authors: *A. MARREIROS, N. LOGOTHETIS, O. ESCHENKO;
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Abstract: The level of norepinephrine (NE) in the brain modulates a variety of cognitive processes such as attention, perception, learning and memory. Stimulation of the Locus Coeruleus (LC), the major source of NE in the forebrain, can change spontaneous and task-related neuronal discharge in a large number of LC projection-targets. Few advances have been done on the study of the effects of phasic NE release on the responsiveness of mPFC cortical areas. In order to have a more complete understanding of the widespread projections of LC we need a multimodal approach. Here, we investigate the effects of LC discharge on ongoing and sensory-evoked cortical activity, by combining LC direct electrical stimulation (LC-DES) with multite site extracellular recordings and whole-brain fMRI in rats under anesthesia. The combination of these methods allows the acquirement of a richer dataset which carries unique insight into the mechanism of large scale NE modulation. The aim of this project is to combine multi-site extracellular recordings, DES and functional MRI techniques in an attempt to define brain “states” and their conditional probabilities with respect to the LC activity level and salient external events. The activity of the noradrenergic system is expected to strongly contribute to the modulation of the cortical state [Neuron 69:1061-1068, 2011]. The cortical recordings, from mPFC, are used to determine the network state prior to sensory stimulation and the neurophysiological responses to sensory stimuli with or without LC stimulation. In order to characterize the different cortical states induced by the anesthesia level and classify its maps accordingly, we computed a cortical synchronization index (SI) proxy [J Neurosci 29: 10600-10612, 2009] using the baseline preceding stimulation. We obtained representations for the distributions of a lower (a) and a higher (b) synchronization index during the same foot-shock (FS) condition. Subsequently, we looked at the cortical state-dependent effect of the FS
stimulation, which shows the Z-score of the BOLD time course difference between the SI distributions. Furthermore, fMRI maps for LC-DES have shown to produce an interesting dichotomy between BOLD responses of cortical and subcortical structures (belonging to metencephalon, mesencephalon and diencephalon cortices). Namely, they show the fraction of positively and negatively activated ROIs for the same LC-DES condition averaged over 10 sessions. This study suggests that it is possible to map the whole brain noradrenergic system by characterizing the FS or LC-DES stimuli responses and contextualize it according to the endogenous cortical synchronization state.

Disclosures: A. Marreiros: None. N. Logothetis: None. O. Eschenko: None.

Poster

512. Sensorimotor Transformation: Higher Order Functional Organization

Location: Hall A

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Topic: D.05. Visual Sensory-motor Processing

Support: HHMI

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The Swartz Foundation

Title: Brain-wide mapping of functional neuron groups in larval zebrafish

Authors: **X. CHEN**¹, Y. MU², Y. HU¹, J. WITTENBACH², J. FREEMAN², F. ENGERT¹, M. B. AHRENS²;
¹Harvard Univ., Cambridge, MA; ²Janelia Res. Campus, Ashburn, VA

Abstract: Simultaneous whole-brain functional imaging should in principle provide a comprehensive understanding of the functional organization of the brain. With recent advances in light-sheet calcium imaging for behaving larval zebrafish (Vladimirov et al., 2014), we are able to record the ~100,000 neurons of the entire brain simultaneously at single-neuron resolution. However, the sheer quantity of neurons in whole-brain data makes extracting such an understanding a daunting task. An important first step is breaking up the neurons into functionally related clusters. These clusters then form manageable units for understanding and modeling the brain. Here we employ a unique combination of regression and clustering
algorithms that classify neurons into functional clusters (~100 clusters per animal). This algorithm identifies several known anatomical regions as well as many others exhibiting previously uncharacterized activity patterns. Using the imaging system developed in Vladimirov et al., 2014, we recorded the activity of practically all neurons simultaneously (at 2 brain-volumes per second), while presenting various visual stimuli (light gradients, moving visual scenes, etc.) and recording fictive swimming behavior. The average activity traces of individual neurons were automatically extracted for the vast majority of neurons in the brain. To obtain brain-wide clustering of neurons based on functional activity, we first had to address two closely related challenges intrinsic to this dataset: (i) the number of clusters is large and unknown; (ii) a large fraction of neurons lack well-defined membership to any cluster. We combined and customized a number of methods to suit this type of data, including both regression and temporal kernel fitting, and developed an automated iterative algorithm to cluster neurons based on the similarity of their activity profiles. Many of the extracted clusters are spatially localized, laterally symmetric and consistent across fish, even though no information of anatomical locations of the cells is used during the clustering. Many of these clusters correspond well to known anatomical regions (e.g. the Raphe nuclei) and other genetically defined cell populations. The collection of these functional clusters ranges from highly sensory-correlated to highly motor-correlated, although many clusters are not directly correlated to either and would have been hard to identify without simultaneous recording of whole-brain activity. This comprehensive collection of clusters allows us to explore large-scale features of brain activity, and provides a basis for further analysis such as inferring functional connectivity.


Poster

512. Sensorimotor Transformation: Higher Order Functional Organization

Location: Hall A

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Topic: D.05. Visual Sensory-motor Processing

Support: NIH R01 EY014924

NIH F32 MH102049

Title: Modulation of the pupil light reflex by frontal eye field microstimulation
Abstract: The primate prefrontal cortex is involved in the flexible control of behavior in response to cognitive processes such as attention. However, the circuit-level mechanisms that underlie this control are unclear. In simpler nervous systems, higher-order control systems sometimes regulate the action of lower-order stimulus-response circuits. However, it remains unclear whether the primate prefrontal cortex can regulate lower-order stimulus-responses. In order to address this question, we asked whether activity in the prefrontal cortex is sufficient to modulate a canonical reflexive stimulus-response: the pupil light reflex. The pupil light reflex (PLR) is a transient pupil constriction elicited by luminance increments that depends on a well-characterized brainstem circuit. Critically, the magnitude of the pupil light response is not entirely determined by the evoking stimulus. Instead, emerging evidence indicates that attention and oculomotor processes modulate the PLR. However, the neurobiological origin of these modulations is unknown. One possible source is the frontal eye fields, a prefrontal area with an established role in attention and gaze control. Therefore, in two monkeys, we measured the effect of low-current microstimulation of the FEF on the PLR. In each experiment, we first evoked saccadic eye movements with microstimulation (100 ms train duration, 10-150 μA, biphasic pulses, 0.2 ms pulse duration, 250-333 Hz). This allowed us to identify the retinotopic location represented by a particular FEF site (“stimulated location”) and its saccadic threshold (< 50 μA). Next, we lowered the current below the saccadic threshold (10-25 μA) and paired FEF stimulation with either flashed visual-probe stimuli that elicited the PLR or with sham stimuli that did not elicit a PLR. Critically, the probe stimuli were either placed within the stimulated location, or outside of it. We found that subthreshold FEF microstimulation (> 25 FEF sites) did not affect pupil size in the absence of probe stimuli. However, FEF stimulation increased the gain of the PLR. It enhanced the pupil response to light probes flashed in the stimulated location and suppressed the PLR outside of it. Thus, the FEF may contribute to the descending modulations of this brainstem reflex.

Disclosures: B.A. Ebitz: None. T. Moore: None.
Support: Schilling Foundation

DFG WI 4046/1-1

DFG Center for Nanoscale Microscopy and Molecular Physiology of the Brain

Title: Comparison of BOLD activity induced by microstimulation of pulvinar and LIP in a behaving monkey

Authors: *L. GIBSON1, M. WILKE2,1, I. KAGAN1;
1Deutsches Primatenzentrum, Goettingen, Germany; 2Cognitive Neurol., Univ. Med. Sch., Goettingen, Germany

Abstract: The thalamic pulvinar and the lateral intraparietal area (LIP) share strong reciprocal anatomical connections and are part of an extensive network of frontoparietal and temporal cortical and subcortical regions, involved in spatial attention and visuomotor planning of eye movements. Our goal was to map the effective connectivity of dorsal pulvinar (dPulv) and LIP and identify their shared circuitry and respective contribution to saccade planning activity. To this end, we applied unilateral electrical microstimulation in the dPulv and LIP in combination with event-related BOLD fMRI in a monkey performing a fixation and a memory-guided saccade task to left or right hemifield targets. Electrical microstimulation (biphasic pulses, 100-250 µA, 200 ms trains at 300 Hz, 1 train per s) was delivered during the 10 s memory period in saccade trials or during the corresponding epoch in fixation trials, interleaved with trials without stimulation. Microstimulation in both dPulv and LIP similarly enhanced task-related activity in frontal areas (e.g. medial FEF, area 46, caudate), in multiple regions along the dorsal bank and fundus of superior temporal sulcus (“sts”: TPO, PGa, FST), and extrastriate cortex. In addition, dPulv stimulation led to activation in lateral FEF, areas 45, 44, PMd, insula and amygdala, while LIP stimulation activated medial parietal area PG. Despite extensive activation along the intraparietal sulcus evoked by LIP stimulation (LIP, VIP, LOP), more posterior LIP sites led to different activity patterns in frontal cortex (area 8B, 46v, but not FEF) and visual areas (PO, V3A) but not in sts. LIP microstimulation induced strong activity in the homologous part of the opposite LIP while for pulvinar stimulation no such homologous activation was found. Nevertheless, both dPulv and LIP stimulation induced activity in several cortical areas in the opposite hemisphere, implying transmission via polysynaptic connections. Most regions did not show a task-dependent stimulation effect: stimulation enhanced task-related activity to a similar extent. Task-dependent stimulation effects (i.e. stronger enhancement during planning of contraversive saccades) were more evident after LIP stimulation, especially in the opposite hemisphere. In conclusion, we identified overlapping and distinct patterns of thalamocortical and corticocortical connectivity of key visuospatial areas, highlighting the dorsal bank and fundus of sts as a prominent shared node. The fact that unilateral dPulv and LIP stimulation exerted major effects on saccade planning activity in both hemispheres may also constrain interpretations of behavioral effects of microstimulation.
**Disclosures:**  

**Poster**

512. Sensorimotor Transformation: Higher Order Functional Organization

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 512.05/O24

**Topic:** D.05. Visual Sensory-motor Processing

**Support:** Canadian Institutes of Health Research  
Canada Research Chairs Program

**Title:** Effector-specific cortical mechanisms for memory-guided reaches and saccades: progression from target memory through motor planning and execution

**Authors:** *D. C. CAPPADOCIA¹, S. MONACO², Y. CHEN¹, J. CRAWFORD¹; ¹Ctr. for Vision Res., York Univ., North York, ON, Canada; ²Ctr. for Mind/Brain Sci., Univ. of Trento, Trento, Italy

**Abstract:** The human brain areas involved in reach and saccade planning have been studied extensively, but the effector-specific cortical mechanisms underlying target representation, motor planning, and motor execution have not been clearly differentiated. In this study, we used an event-related fMRI design that temporally separated the major stages of memory-guided reaching and saccades into three distinct phases: visual target representation, motor planning, and motor execution. In each trial, subjects (N=12) fixated at midline and were briefly shown a target located between 4-7 degrees to the left or right of the midline. After a delay of 8 seconds (the effector-independent target representation phase), subjects were instructed with an auditory cue to perform a reach or a saccade. This was followed by a second delay of 8 seconds (the effector-specific motor planning phase). Finally, an auditory ‘go signal’ prompted subjects to perform the instructed movement by reaching-to-touch a touchscreen with their right hand or performing a saccade (the effector-specific motor execution phase). In a control condition, subjects indicated the colour of the initial target. Our analysis to date (N=5) has focused on effector specificity in the motor planning and execution phases, i.e., cortical areas that show a preference for either reach or saccade tasks. This preliminary analysis indicates that during motor planning, bilateral dorsomedial parietal, M1, S1, and dorsal premotor areas show a preference for reach planning over saccades. During motor execution, the same areas contributed to a preference for reaches, while bilateral parietal areas (dorsolateral posterior parietal cortex and angular gyrus) and the right inferior frontal gyrus showed a preference for saccades. These preliminary results indicate...
that a cortical preference for reaches emerges during movement planning, whereas planning for saccades only emerges during motor execution.

**Disclosures:** D.C. Cappadocia: None. S. Monaco: None. Y. Chen: None. J. Crawford: None.

**Poster**

512. Sensorimotor Transformation: Higher Order Functional Organization

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 512.06/O25

**Topic:** D.05. Visual Sensory-motor Processing

**Support:** BioHybrid Human Network, The University of Sheffield

**Title:** Reduction of cortical activity during task learning reflects representation efficiency in the motor cortices

**Authors:** *G. SPIGLER*¹, R. TIMMERS², S. WILSON²;
¹Dept. of Psychology, ²The Univ. of Sheffield, Sheffield, United Kingdom

**Abstract:** In the present work we propose a biologically plausible framework for action learning in the motor cortex in the context of sensori-motor tasks. We then validate it against data on Repetition Suppression, that is the observed phenomenon of reduction in cortical activity after task learning and sensory priming. Within this framework, we assume that the reduced activity is due to learning of a specialized, efficient representation of motor patterns that are useful for the individual in the context of the task. We hypothesize that prior to training on a complex motor task, a large set of neurons is recruited in order to produce the desired novel actions, which is reflected in a widespread activation. After learning, however, a smaller specialized pool of neurons is sufficient to produce the desired task-specific actions, resulting in a lower overall activity. For the scope of our project, music is especially well suited as it is possible to measure how the brain is affected during musical training on naïve subjects that have no prior experience in music, and thus lack any neural representation of the actions involved. Finally, we investigate the role of our model in the context of embodied cognition, preparing future work on a humanoid robot platform.

**Disclosures:** G. Spigler: None. R. Timmers: None. S. Wilson: None.
Title: Prosthetic limb usage relates to increased visuo-motor functional coupling and enhanced visual processing of prosthetic limbs in hand-selective cortical regions

Authors: F. M. Z. VAN DEN HEILIGENBERG¹, T. ORLOV², S. MACDONALD³, E. P. DUFF¹, D. HENDERSON SLATER⁴, H. JOHANSEN-BERG¹, J. C. CULHAM³, *T. R. MAKin¹;
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Abstract: Regions in the lateral occipitotemporal cortex (LOTC) selectively respond to images of hands and arms. The functional role of these regions in supporting motor control is unknown. Arm amputation introduces a change in individuals' ability to interact with their environment and in brain organisation. Using a prosthetic limb to compensate for hand loss strongly depends on both motor control and visual information, especially considering the lack of somatosensory inputs. Here we studied how prosthesis usage affects connectivity and activity in visual and motor hand-selective areas in 32 individuals with acquired or congenital upper limb loss. To study changes in coupling between the visual and the motor systems, we analysed resting-state functional connectivity. We placed a seed region in the territory of the missing hand in primary sensorimotor cortex (deprived cortex). We predicted that prosthesis usage in daily life should result in increased coupling between visual and motor hand-selective regions. We found a significant positive correlation between prosthesis usage and functional connectivity between the deprived cortex and visual hand-selective voxels in LOTC. We suggest that the increased connectivity between visual and motor hand-selective regions might result from coordination between these two regions during prosthesis usage. We predicted that usage-based changes in connectivity may also affect the way hand-selective visual regions respond when participants view their own and others' prostheses. We found a positive correlation between prosthesis usage and activation levels in the same LOTC region that showed experience-dependent connectivity changes. Moreover, the statistical relationship between prosthesis usage and prosthesis representation in LOTC was mediated by increased functional coupling, as assessed during rest. Our findings suggest that prosthetic limb usage can shape how high-level visual cortex interacts...
with sensorimotor cortex to represent visual information. These results suggest that LOTC connectivity and function is influenced by visuomotor control. Visual hand-selective regions may therefore play a role in supporting action execution. Furthermore, these findings provide insight into the mechanisms underlying the mechanisms of successful prosthesis usage, which may aid in developing neurorehabilitation strategies.


**Poster**

**512. Sensorimotor Transformation: Higher Order Functional Organization**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 512.08/O27

**Topic:** D.05. Visual Sensory-motor Processing

**Support:** NSERC-Natural Sciences and Engineering Research Council of Canada

CIHR-Canadian Institutes of Health Research

**Title:** Functional modulation of corticospinal excitability in motor mirror neurons after observational skill learning

**Authors:** *M. VESIA, R. PELLICCIARI, R. F. CASH, R. ISAYAMA, R. CHEN; Univ. of Toronto, Toronto Western Res. Inst., Toronto, ON, Canada

**Abstract:** Background: Action observation of skill learning can lead to plastic changes at the motor system level. However, it remains unclear whether observing repetitive actions, in the absence of skill learning, modulates excitability of the human primary motor cortex (M1). We hypothesized that observing skilled motor learning not only improves behavioural performance in the untrained right-hand, but also enhances excitability of the motor representation for the action. By contrast, we hypothesized that observation of simple repetitive movements, in the absence of skill learning, produces no plastic changes in M1. Methods: Eight right-handed subjects were studied. We used transcranial magnetic stimulation (TMS) to probe cortical spinal excitability (CSE) adaptations associated with observing a video depicting another person: (1) learning a sequence-specific serial reaction-time task (SRTT) with the right hand; (2) performing a repetitive, right-hand movement on a random-sequence SRTT; and (3) learning a sequence-specific SRTT with no action (i.e., viewed inactive right-hand). Before and immediately after
each experimental condition, we measured behavioural performance (speed and accuracy of untrained right-hand) and CSE in motor mirror neurons with TMS. These include resting motor threshold (RMT) and motor evoked potential (MEP) amplitudes bilaterally. Results: We found learning by action observation (condition 1) not only decreased reaction time in the SRTT task in the untrained right-hand, but also increased M1 excitability in the left hemisphere. These changes were selective for learning by observation, as they did not occur after observing performance of the random sequence (condition 2). We also found that, although learning by observation modulates offline-resting CSE in M1, there were differences in the pattern of motor cortical plasticity when learning a repeating sequence structure with versus without motor action. Goal-based learning (condition 3) increased offline-resting CSE in M1 bilaterally, whereas learning by action observation (condition 1) was lateralized to the internal model of the body that maps the motor commands necessary to produce the overt movement. Conclusion: We conclude that the acquisition of a novel motor skill is a prerequisite factor in driving neurophysiologic changes in motor mirror neurons after action observation. We also show that the motor-memory formation is modulated by visual feedback of the action, suggesting a strong link between sense of agency and action outcome.


Poster
512. Sensorimotor Transformation: Higher Order Functional Organization

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 512.09/O28

Topic: D.05. Visual Sensory-motor Processing

Support: I-CORE Program of the Planning and Budgeting Committee,

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Sagol School of Neuroscience

Title: Encoding action affordances during passive observation of graspable objects
Authors: *S. SIMON*1,2, R. GILRON1,2, R. MUKAMEL1,2,
1Sagol Sch. for Neurosci., 2Sch. of Psychological Sci., Tel Aviv Univ., Tel Aviv, Israel

Abstract: In our daily life we constantly interact with various objects in the environment. Ethological perspectives view this interactive behavior as a result of an online selection process from different potential motor actions that can be made towards objects within reach. At the neural level, such potential actions can be simultaneously represented by different populations of visuo-motor cells. Canonical neurons, a subset of motor cells that are found in various regions of the motor system, respond during passive observation of graspable objects without an interacting agent. These neurons have the functional properties to support the representation of possible motor plans that are afforded by adjacent objects (e.g. action affordances). However, whether these neurons differentially encode specific action affordances triggered by different objects is not known. To address this issue we recorded whole-brain fMRI on nine healthy right handed subjects. The subjects passively viewed images of a mug with a handle positioned to the right or the left (i.e. affording a left or right hand grip orientation respectively). We performed whole brain searchlight Multi-Voxel-Pattern-Analysis (MVPA) to compare the spatial patterns of activity across multiple voxels differentiating the orientation of observed mug. Passive observation of mugs with identical grip orientation but different colors served as a control to the analysis. We found that spatial activity patterns in the left dorsal pre-motor cortex, left thalamus and bilateral primary visual cortices were selective to mug grip orientation with classification accuracy of 65% (*q*<0.01, FDR corrected). Importantly, these regions could not decode the color of the mug above chance level suggesting that low-level visual cues are not the source of this differentiation. Our results support the notion that passive observation of graspable objects, elicits a neural representation of specific action affordances that are encoded differently within motor and visual regions.

Disclosures: S. Simon: None. R. Gilron: None. R. Mukamel: None.

Poster

512. Sensorimotor Transformation: Higher Order Functional Organization

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 512.10/O29

Topic: D.05. Visual Sensory-motor Processing

Support: ERC parietalaction

Title: Seeing another speak actives Spt and neighboring parietal area PFm
Authors: *D. CORBO, G. ORBAN;
Univ. of Parma, Parma, Italy

Abstract: Several regions of posterior parietal cortex are specifically activated by observing classes of actions (e.g., locomotion, manipulation, skin-displacing actions) performed by others and they are supposedly involved in the sensorimotor transformations for planning those actions. For technical reasons, it is often impossible to compare action observation with action execution. To establish such a match, 22 human volunteers were presented video-clips showing an actor, viewed from the side, performing one of four action classes, each with four exemplars: mouth communication actions (speak, sing, shout, and whistle) directed to a conspecific (communicative actions), mouth actions directed towards fruits (oral actions), hand actions directed towards objects (manipulative actions), both mouth and hand actions directed towards fruits (mouth & hand actions). Two different types of control stimuli were used: static images taken from the action videos, and ‘dynamic scrambled’ stimuli derived by temporally scrambling a noise pattern animated with the motion extracted from the original action video. In a different session the same volunteers mentally rehearsed “jabberwocky” sentences to localize area Spt in left parietal cortex, described by Hickok and colleagues (JCNS 2003). A Spt ROI was defined in individual subjects in three steps. First the contrast rehearse vs rest (at p<0.001 as a threshold) was computed for each subject. Second, we looked for the local maxima nearest to the left Spt site according the coordinate of Hickok (-50, -48, 28). Finally, we built a 27 voxel ROI around the individual maxima. An ANOVA investigating the effects on the Spt activation of the factor type of action reached significance (p<0.01). Particularly the Spt activation was stronger for communicative action observation than any other. In addition, in a whole brain analysis we defined the specific statistical maps for each action class, using inclusive and exclusive masking procedures. The specific map for oral communication observation included a parietal site located in left PFm, in addition to left BA45, and bilateral STS sites. The specific maps for manipulative, oral, mouth, and hand actions included as expected phAIP. These results confirm that the same PPC regions are activated by both execution and the observation and suggest additional parietal regions specifically involved in the observation of a given action class. Supported by ERC parietalaction

Disclosures: D. Corbo: None. G. Orban: None.

Poster

512. Sensorimotor Transformation: Higher Order Functional Organization

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 512.11/O30
Title: Neural mechanisms of body language: Does body language share common neural mechanisms with vocal language?

Authors: *Y. SATO, A. MATSUO, S. MORIOKA; health science, Kio Univ., Kitakatsuragi-Gun, Japan

Abstract: [Purpose] Humans communicate with each other not only using verbal language but also body language. Prior studies reveal that Broca’s area is involved in verbal expression; however, the neural mechanisms of body language are unclear. Several cognitive psychology studies assume that verbal and body language have a common underlying mechanism prior to expression. A method to analyze effective connectivity in the brain has recently been established. We applied this method to discriminate between the neural mechanisms of body language and verbal language.

[Methods] Eight subjects participated in this experiment. Electroencephalogram (EEG) data were recorded from 64 channels using the BioSemi Active Two system and a sampling frequency of 512 Hz. The processing was done using an EEGLAB tool box and SIFT. Subjects were seated in front of a computer screen and shown pictures that implied “big” or “small” (e.g., big bear or small butterfly). After seeing every picture, subjects were instructed to indicate their judgment using verbal language (Experiment 1) or hand gestures (Experiment 2). In the control condition, subjects were instructed to do nothing during seeing same pictures. EEG data were sampled during these tasks.

[Results & Discussion] Although subjects saw the same pictures in both verbal language and hand gesture tasks, different effective connectivity (EC) was induced during the cognitive process prior to the expression. The network hub was located at the tempoloparietal junction in the former condition and at the fusiform gyrus in the later. In addition, we found difference of pass in EC after 150 msec of stimulus onset. These results indicated that behavioral tasks influenced the neural process of cognition and changed EC.

Disclosures: Y. Sato: None. A. Matsuo: None. S. Morioka: None.
Title: Investigating interhemispheric transmission with tRNS

Authors: J. R. MCINTOSH1,2, C. MEHRING1

Bernstein Ctr. Freiburg & Fac. of Biol., Freiburg Univ., Freiburg, Germany; 2Bioengineering, Imperial Col. London, London, United Kingdom

Abstract: The Poffenberger paradigm (Poffenberger, 1912) demonstrated that when healthy subjects are asked to react to a stimulus with the same hand as the side of the visual hemifield in which it appears (uncrossed condition), their response times are faster than when their reaction must occur on the opposite side (crossed condition). This finding has been interpreted as being caused by information having to transfer via the corpus callosum in the crossed condition, consequently increasing reaction times. Here, we attempt to interact with this effect using transcranial random noise stimulation (tRNS). Our paradigm was based on a modification of the Poffenberger paradigm. Subjects sat in front of a monitor while fixating a cross placed in its centre. At brief randomised intervals in the range 1.8-3.2s, subjects are displayed a coloured target in either their left or their right visual field. The subject's task is to respond as quickly to the target colour as possible, pressing a key with their left hand for one colour, and with their right hand for the other, while ignoring the physical location of the target. Simultaneously, 40s tRNS blocks are interleaved with 40s sham blocks and applied via sponge electrodes positioned at FT7 and FT8 on the EEG 10-20 system. Response times to the appearance of the visual target were recorded and analysed. During the sham condition, as in the standard Poffenberger paradigm we find cross condition response times to be significantly slower than in the uncrossed condition. Our results are also consistent with a facilitation of communication between the two hemispheres in the crossed condition being caused by stimulation. More specifically however, a decrease in response time is apparent in the left-visual field to right hand condition, and not in the opposite, right-visual field to left hand condition. Via a meta-analysis of experiments utilising the Poffenberger task, Marzi et al. (1991) have shown that there is an asymmetry in response time between the two crossed conditions, with the left-visual field to right hand condition producing quicker responses. Consequently, a potential explanation for our results may be that via stochastic facilitation, tRNS enhances this inherent asymmetry. Poffenberger, A T. (1912). Reaction time to retinal stimulation with special reference to the time lost in conduction through nervous centers. Archives of Psychology, 23, 1-73. Marzi, C. A, Bisiacchi, P., & Nicoletti, R. (1991). Is interhemispheric transfer of visuomotor information asymmetric? Evidence from a meta-analysis. Neuropsychologia, 29(12), 1163-1177.

Disclosures: J.R. McIntosh: None. C. Mehring: None.

Poster

513. Inflammatory Pain
Title: Chronic inflammation causes reduced peripheral drive in primary afferents in both young and aged mice

Authors: *A. Weyer*, C. L. O'Hara, C. Stucky; 
1Cell Biol., 2Cell Biology, Neurobiology, and Anat., Med. Col. of Wisconsin, Milwaukee, WI

Abstract: It has long been known that peripheral inflammation causes enhanced pain sensitivity at the behavioral level and increased action potential generation in nociceptive primary afferents in rodent models. However, few studies have examined the effects of chronic inflammation (> 4 weeks) on pain behaviors in these animals, and fewer still have explored whether increased peripheral drive still contributes to pain sensation at this time point. Additionally, despite the significant pain epidemic in elderly human populations throughout the world, little basic science research has elucidated whether different pain mechanisms occur in aged animals as compared to young animals. Therefore, we sought to determine the contribution of primary afferent neurons in generating pain behaviors during both the acute and chronic phases of inflammation in both young and aged animals. We found that both young (2 months) and old (> 18 months) mice exhibited reduced paw withdrawal thresholds in response to mechanical stimuli following both acute (2 days) and chronic (8 weeks) Complete Freund’s Adjuvant-mediated inflammation. Interestingly, however, young mice exhibited more behavioral sensitization to mechanical stimuli than their aged counterparts throughout the entire inflammatory process. Although young and old mice exhibited differences in their behavioral responses to acute and chronic inflammation, their primary afferents innervating the inflamed region responded with similar action potential firing rates in response to a series of increasing mechanical forces. This indicates that the difference in behavioral mechanical sensitivity following chronic inflammation is likely due to differences in central mechanisms in young and aged animals, and is unlikely to be due to differences in peripheral drive. As has been shown previously, action potential firing rates to mechanical stimuli were elevated following acute inflammation (2 days) in both young and aged animals as compared to controls. Surprisingly, however, we found that action potential firing rates from C fiber nociceptors in inflamed animals were significantly reduced as compared to controls at 8 weeks in both young and aged animals. This suggests a novel plastic change in peripheral sensory afferents that dampens their responses in order to limit pain transmission to
the central nervous system after chronic (8 weeks) inflammatory injury. Collectively, these results argue that acute inflammatory insult causes peripheral sensitization in C fiber nociceptors to generate pain sensation, but that pain behavior due to chronic inflammation is largely a central phenomenon in both young and aged animals.

Disclosures: A. Weyer: None. C.L. O'Hara: None. C. Stucky: None.

Poster

513. Inflammatory Pain

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 513.02/O33

Topic: D.08. Pain

Support: NS072432

Title: Mechanisms of complement C5a-induced mechanical sensitization in mouse: The roles of macrophages, cytokines and TRPV1

Authors: *C. WARWICK¹, L. P. SHUTOV¹, Y. M. USACHEV³, D. J. CLARK², X. SHI³;
¹Pharmacol., Univ. of Iowa, Iowa City, IA; ²Dept. of Anesthesia, VA Palo Alto Healthcare Syst. and Stanford Univ., Palo Alto, CA

Abstract: The complement system is a principal component of innate immunity. It consists of more than 30 proteins that are rapidly recruited through a cascade of enzymatic reactions to contribute to host defenses through diverse mechanisms. In spite of growing evidence implicating the complement system in the development of pain hypersensitivity, the underlying mechanisms are not understood. We found that injection of 500 ng C5a into a mouse hind paw produced strong mechanical sensitization that lasted for at least 3 hrs, and fully recovered to pre-injection levels within 24 hrs. Interestingly, this hyperalgesia produced by C5a was blocked by the TRPV1 antagonist AMG9810 and was strongly reduced in TRPV1 KO mice suggesting a possible role for neurogenic inflammation. Examination of inflammatory mediators in the skin induced by intraplantar C5a injection showed an increase in the levels of numerous cytokines including CCL2. C5a-induced mechanical sensitization was reduced in CCL2 KO mice. Immunohistochemical examination of plantar skin sections revealed that C5aR was expressed primarily in resident skin macrophages. Ca2+ imaging in cultured mouse primary macrophages demonstrated that C5a induced a large elevation in intracellular Ca2+ concentration ([Ca2+]i) in these cells. C5a-induced [Ca2+]i responses were blocked by the Gβγ inhibitor gallein and phospholipase C inhibitor U73122. We also found that drug-induced macrophage depletion in
transgenic MAFIA (macrophage Fas-induced apoptosis) mice reduced C5a-induced mechanical sensitization. We propose that the complement fragment C5a triggers macrophage-to-neuron signaling that involves CCL2 and TRPV1, which ultimately leads to mechanical sensitization.

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Poster

513. Inflammatory Pain

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

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Topic: D.08. Pain

Support: GACR15-111388

MSMT LH12058

GACR P304/12/G069

CZ.1.07/2.3.00/30.0025

CZ.1.05/1.1.00/02.0109

RVO67985823

Title: Modulation of nociceptive synaptic transmission by PAR2 receptors at spinal cord level in a model of peripheral inflammation

Authors: *P. MRÓZKOVÁ, J. PALEČEK;
Inst. of Physiol. Acad. of Sci., Prague, Czech Republic

Abstract: Modulation of synaptic transmission in the spinal cord dorsal horn plays a key role in the development of pathological pain states and chronic pain diseases. Protease-activated receptors (PARs) are a family of four G-protein-coupled receptors (PAR1-4) activated by proteases. The role of PAR2 receptors in pain perception is well established in the peripheral tissues. However, the role of PAR2 receptors on the central branches of DRG neurons in the spinal cord is not fully understood. The present study aimed to study the role of PAR2 receptors in nociceptive processing and modulation of synaptic transmission in the superficial dorsal horn (DH) neurons in a model of carageenan induced peripheral inflammation. Whole-cell patch clamp recordings of miniature - mEPSCs and spontaneous - sEPSCs were made from superficial
DH neurons in acute spinal cord slices prepared from Wistar rats 21 days old, in the presence of strychnine (5uM) and bicuculline (10uM), at -70mV holding potential. TTX (0.5uM) application was used for mEPSC detection. Peripheral inflammation was induced by application of 3% mixture of carrageenan and kaolin into the paw 24h before the experiment. Application of PAR2 agonist SLIGKV-NH2 (PAR2 AP, 100µM) in slices from naive animals induced inhibition of miniature excitatory post-synaptic current frequency from the control level (60,0±4,3; % n=20; p<0,001), while under the inflammatory conditions it induced increase of mEPSC frequency (121,3±9.3%; n=17; p<0,01). This effect of the PAR2 agonist application was significantly different between the naive and inflammatory groups (p<0,001). PAR2 AP application induced spontaneous EPSC frequency increased in both the naive (122,9±6,0; n=18; p<0,01) and inflammatory conditions (137,8±15.1%; n=15; p<0,01). Inactive peptide VKGILS-NH2 (100uM) application did not evoke any change in the mEPSCs and sEPSC frequencies. Each neuron was tested for the presence of capsaicin (0.2 uM) evoked response in the end of the experiment. Out of the 70 neurons tested in this study 86% of them increased frequency of EPSC after the capsaicin application. Our results suggest that presynaptic PAR2 receptors may play an important role in modulation of nociceptive synaptic transmission in the spinal cord dorsal horn particularly under conditions of peripheral inflammation. Further experiments are needed to fully evaluate the role of spinal cord PAR2 receptors in pain modulation.

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Poster

513. Inflammatory Pain

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Topic: D.08. Pain

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Title: Neuronal gene therapy of murine carrageenan-induced inflammatory pain with human carbonic anhydrase-8 using AAV8 virus

Authors: *G. Z. ZHUANG1, B. KEELER1, J. GRANT2, L. BIANCHI2, D. M. ERASSO1, A. S. PANTRY1, S. BANDREMER1, E. S. FU1, W. TIM3, K. D. SARANTOPOULOS1, L. DIATCHENKO4, S. SMITH4, W. MAIXNER4, E. R. MARTIN5,6, R. C. LEVITT1,5,6,7;

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Carbonic anhydrase-8 (CA8) is an allosteric inhibitor of inositol trisphosphate receptor-1 (ITPR1), which regulates neuronal intracellular calcium release. Calcium dysregulation is causally linked with chronic pain. Previously, we reported that the mouse ortholog Car8 was also involved in pain regulation. Here we investigate whether overexpression of human CA8 (hCA8) using sciatic nerve injection of AAV8-hCA8 virus with a C-terminal V5 tag induces analgesia and inhibits hyperalgesia in a murine inflammatory pain model. Our pain behavior data show that overexpression of hCA8 in waddle mice, a naturally occurring Car8 null-mutant mouse (MT), completely reversed mechanical and thermal hyperalgesia. Our immunohistochemistry and western blot data show that overexpression of hCA8 protein inhibited forskolin-induced ITPR1 phosphorylation (pITPR1) in vitro; and in lumbar DRG of MT mice after sciatic nerve gene transfer of AAV8-hCA8 viral particles. Eighty-three percent of V5-positive DRG cells were small to medium-sized neurons (<700 µm²), suggesting AAV8-hCA8 primarily infected nociceptive neurons. Double Immunofluorescence shows V5-positive cells co-localized with calcitonin gene-related peptide, substance P and Isolectin B4 in lumbar DRG. Horizontal sections of the lumbar spinal cord show V5-immunoreactive afferent fibers mainly within the superficial lamina I and II of the dorsal horn (DH), and colocalized with the neuronal marker, Tuj1. Many of these V5-positive afferents were highly branched and longitudinally located. V5-positive structures were also observed in DRG satellite cells and sciatic nerves (SN) Schwann cells. In addition, we showed hCA8 overexpression in vitro significantly inhibited ATP-induced cytoplasmic free calcium release. Finally, we demonstrate that hCA8 overexpression produces analgesia and anti-hyperalgesia in carrageenan sub-acute inflammatory pain models. Our data show that overexpression hCA8 in the DH, DRG and SN may regulate chronic pain through mouse peptidergic and non-peptidergic neurons, as well as glial cells. These findings suggest that hCA8 can be potentially used to treat chronic inflammatory pain through regulating the activity of ITPR1 and free intracellular calcium levels in critical nociceptive pathways.


Poster

513. Inflammatory Pain

Location: Hall A
Title: Phosphatidylinositol 3-kinase and phospholipase C mediate Epac-induced sensitization of rat sensory neurons

Authors: *B. SHARIATI¹, G. D. NICOL²,³, M. R. VASKO²,³,⁴
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Abstract: We have previously demonstrated that activation of exchange proteins directly activated by cAMP (Epacs) sensitizes rat sensory neurons in a Ras-dependent manner. Because Ras has a number of downstream effectors, the question remains as to which signaling pathway(s) mediate the Epac-induced increase in sensitivity of sensory neurons. To examine this question, we studied whether selective inhibitors of phosphatidylinositol 3-kinase (PI3K), phospholipase C (PLC), or extracellular signal-regulated kinase (ERK) could alter the ability of the Epac agonist 8CPT-AM to augment action potential (AP) firing or capsaicin-evoked release of immunoreactive calcitonin gene-related peptide (iCGRP) from adult rat sensory neurons grown in culture. Pretreating sensory neuronal cultures for 30 min with 3 µM of the PI3K inhibitor, LY294002, blocked the ability of 8CPT-AM to increase APs generated by a ramp of current. This inhibitor also attenuated the ability of 8CPT-AM to augment capsaicin-evoked iCGRP release. In the presence of the inactive analog, LY303511, 8CPT-AM increased release with an EC50 of 2.7 µM, whereas in the presence of the PI3K inhibitor, LY294002, the EC50 for the Epac agonist was 7.9 µM. Pretreating sensory neurons with a selective inhibitor of PI3K gamma also blocked Epac-induced sensitization as measured by an increase in APs and an augmentation of capsaicin-evoked release of iCGRP, whereas, the selective PI3K alpha or PI3K beta inhibitors were ineffective. Exposing neuronal cultures for 30 min to 3 µM of the PLC inhibitor, U73122, did not alter the ability of 3 µM 8CPT-AM to enhance AP firing, but shifted the EC50 of 8CPT-AM to augment capsaicin-evoked release of iCGRP from 2.7 µM (in cells treated with 3 µM of the inactive analogue, U73343) to 3.5 µM in the presence of the PLC inhibitor. Pretreating neurons for 30 min with 3 µM of the mitogen-activated protein kinase kinase (MEK) inhibitor, U0126, did not alter the ability of the Epac agonist to increase ramp-evoked AP firing and did not affect the Epac-induced augmentation of iCGRP release. The EC50 of 8CPT-AM to augment capsaicin-evoked release was 2.5 µM in cultures pretreated with U0126, which was not statistically different from an EC50 value of 3.2 µM in untreated cultures. This concentration of U0126 blocked the ability of the Epac agonist to enhance ERK phosphorylation, demonstrating that it was sufficient to block MEK activity. These results...
demonstrate that the gamma isoform of PI3K is a critical downstream effector of Epac-mediated sensitization of sensory neurons as measured by two endpoints. In contrast, blocking PLC likely alters the ability of Epac to enhance exocytosis independent of AP firing.

**Disclosures:** B. Shariati: None. G.D. Nicol: None. M.R. Vasko: None.

**Poster 513. Inflammatory Pain**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#: 513.06/O37**

**Topic:** D.08. Pain

**Support:** JSPS KAKENHI Grant Number 26460709

**Title:** Expression of NIPSNAP1, a neuropeptide nocistatin-interacting protein, following inflammatory pain

**Authors:** *E. OKUDA-ASHITAKA*¹, K. OKAMOTO¹, M. OHASHI¹, K. OHNO¹, T. MINAMI², S. ITO³; ¹Osaka Inst. of Technol., Osaka, Japan; ²Osaka Med. Col., Takatsuki, Japan; ³Kansai Med. Univ., Hirakata, Japan

**Abstract:** The exclusive expression of 4-Nitrophenylphosphatase domain and non-neuronal SNAP25-like protein homolog (NIPSNAP) 1 is observed in the neurons. The levels of NIPSNAP1 protein and mRNA are changed by several neuronal diseases, including seizures, prepulse inhibition, and phenylketonuria. We previously reported that NIPSNAP1 interacts with the neuropeptide nocistatin (NST), and it is implicated in the inhibition of tactile pain allodynia induced by NST. NST also inhibits some inflammatory pain responses induced by peripheral injection of formalin and carrageenan. In the present study, we examined the gene expression of NIPSNAP1 in inflammatory pain model to determine the role of NIPSNAP1 to inflammatory pain. NIPSNAP1 mRNA was expressed in small- and medium-sized neurons of the dorsal root ganglion (DRG) and motor neurons of the spinal cord but not in a peripheral tissue paw. In the formalin test, nociceptive behavioral response increased in phase II, particularly during the later stage (26-50 min) in NIPSNAP1-deficient mice, and phosphorylation of extracellular signal-related kinase was enhanced at 5 and 30 min in the spinal dorsal horn of the deficient mice, suggesting that NIPSNAP1 deficiency induced the aggravation of inflammatory pain through central sensitization elicited by the formalin test. NIPSNAP1 mRNA level increased from 5 min to 60 min after formalin injection in DRG and reached 1.7-fold increase at 60 min. Moreover,
oral administration of aspirin completely inhibited nociceptive responses induced by formalin in NIPSNAP1-deficient and wild-type mice. Prostaglandin E₂ stimulated NIPSNAP1 mRNA expression via the cAMP-protein kinase A signaling pathway in primary DRG cells. The increased NIPSNAP1 mRNA expression is likely involved in the attenuation of inflammatory pain. In contrast, NIPSNAP1 mRNA levels in DRG were decreased by approximately 40% during 24-48 h after carrageenan injection. The prolonged inflammatory pain induced by carrageenan and complete Freund’s adjuvant was exacerbated in NIPSNAP1-deficient mice, suggesting that the decreased NIPSNAP1 expression is involved in the exacerbation of prolonged inflammatory pain. These results suggest that changes in NIPSNAP1 expression may contribute to the pathogenesis of inflammatory pain.


Poster

513. Inflammatory Pain

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 513.07/O38

Topic: D.08. Pain

Support: National Natural Science Foundation of China Grants (81070888, 81230025 and 81271231)

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Jiangsu Provincial Six Talent Summit Action Plans

Title: DNA hydroxymethylation by Tet1 and Tet3 regulates chronic inflammatory pain

Authors: *Z. PAN¹, Z. XUE², L. HAO², Q. TANG², M. ZHANG², X. YANG², Y. LI², J.-L. CAO²;
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Abstract: The ten-eleven-translocation (Tet) family of methylcytosine dioxygenases converts methylcytosine (5mC) to 5-hydroxymethylcytosine (5-hmC), which plays an important role in regulation of gene transcription. 5hmC and Tet proteins are enriched in mammal central nervous
system. However, their functional regulatory role in chronic pain remains elusive. Here, we found a marked increase of 5hmC content and of Tet1 and Tet3 expression in spinal neurons in complete Freund’s adjuvant (CFA)-induced chronic inflammatory pain mice. Knockdown of spinal Tet1 or Tet3 prevented and reversed thermal hyperalgesia and mechanical allodynia, and spinal neuronal sensitization induced by CFA. The genome-wide analysis revealed that the promoter region of Stat3, containing multiple CpG dinucleotides, is hyperdemethylated underlying chronic inflammatory pain status. Knockdown of spinal Tet1 or Tet3 reduced the increase of 5hmC in Stat3 promoter and Stat3 expression induced by CFA. In contrast, overexpression of Tet1 or Tet3 in naïve mice induced pain-responsive behaviors, which was accompanied with the increase in 5hmC content of Stat3 promoter and in Stat3 expression level. Together, these demonstrate a novel epigenetic mechanism of chronic pain that DNA hydroxymethylation by Tet1 and Tet3 regulates chronic inflammatory pain by targeting Stat3 in spinal cord.

Disclosures: Z. Pan: None. Z. Xue: None. L. Hao: None. Q. Tang: None. M. Zhang: None. X. Yang: None. Y. Li: None. J. Cao: None.

Poster

513. Inflammatory Pain

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Topic: D.08. Pain

Support: CECTI 07-2014

CIC-UMSNH. 26.10

CIC-UMSNH. 30.2

Title: Toluene pronociceptive effect is increased after repeated exposures but is reduced by metamizol in the rat formalin test

Authors: *L. F. ORTEGA-VARELA*, E. ALFARO-PEDRAZA, C. CERVANTES-DURÁN, M. Y. GAUTHHEREAU-TORRES

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Abstract: Toluene can be found in products like thinner, paints and adhesives. Toluene misuse is an important public health problem in Mexico, mainly among street children and teenagers. This solvent shares actions with CNS depressants and several mechanisms have been proposed for its effect. In vitro studies demonstrate that toluene inhibits NMDA receptors and, since NMDA receptors have been implicated in pain, it was initially proposed that this solvent could have involved in neural processes like modulation of pain transmission. However, in vivo studies indicate that toluene produces pronociceptive effects in acute pain models in mice, by mechanisms other than blockade of glutamatergic system activity and it is unclear which neurotransmitter systems could have been participating in pronociception induced by toluene. In order to find out the participation of toluene on nociception in rats after acute or subacute exposure, we used metamizol (a wide-used non-steroidal anti-inflammatory drug with several mechanisms of action). Female Wistar rats (200-300 g) were placed in a static exposure chamber and exposed to toluene (6000 ppm) or air (control group) during 30 minutes (acute exposure) or 30 minutes twice a day during 7 days (subacute exposure). Other groups of rats received metamizol (600 mg/kg, p.o.) and then were exposed to toluene or air as described above. After acute or subacute exposure, rats were injected with 50 µl of 1% formalin in the dorsal surface of the right hind paw. This substance causes a typical flinching pain related behavior, the decrease in the number of flinches reflected as reduction of the area under curve (AUC) is considered as antinociception. We observed that toluene produced a significant increase in AUC compared with rats only exposed to air, both in acute and subacute exposure (568.5 ± 31.4 in toluene group vs. 445.4 ± 7.8 in control group [acute exposure] and 666.7 ± 14.2 in toluene group vs. 468.8 ± 7.5 in control group [subacute exposure]), indicating a pronociceptive effect, that was more pronounced after repeated exposure. On the other hand, metamizol showed an antinociceptive effect (AUC = 244.2 ± 7.9 [acute exposure] and 258.8 ± 5.8 [subacute exposure]) that can counteract toluene pronociceptive effect (AUC = 307.5 ± 6.8 [acute exposure] and 305.8 ± 6.7 [subacute exposure]). These results suggest that toluene repeated exposure increases its pronociceptive effect in rat formalin test; in contrast, metamizol may affect pronociception induced by toluene. This effect could be mediated through common molecular targets between toluene and metamizol.


Poster

513. Inflammatory Pain

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 513.09/O40
**Topic:** D.08. Pain

**Support:** Ministere de l'Enseignement Superieur, de la Recherche, de la Science et de la Technologie - MESRST : PSR-SIIRI-855 Award

Canadian Institues of Health Research- CIHR- MOP 130285

**Title:** Antinociceptive properties of selective melatonin MT2 receptor partial agonists

**Authors:** *G. GOBBI*, M. LOPEZ-CANUL1,2, S. COMAI1, S. DOMINGUEZ-LOPEZ1, V. GRANADOS-SOTO3;
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**Abstract:** Background: Melatonin (MLT) is a neurohormone acting through MT1 and MT2 receptors that has been involved in the regulation of acute and chronic pain likely through its activation of MT2 receptors. We have recently demonstrated that selective MT2 receptor partial agonists have antiallodynic properties in animal models of neuropathic pain by modulating ON/OFF cells of the descending antinociceptive system. Here, we further examined the antinociceptive/antinflammatory properties of the selective MT2 receptor partial agonists UCM765 and UCM924 in two animal models of acute/inflammatory pain: the hot plate and the formalin tests. Methods: UCM765 and UCM924 (5-40 mg/kg) were injected subcutaneously in male Wistar rats 30 min prior to the hot plate or the formalin tests. The effects of UCM765 and UCM924 were compared to those of melatonin (150 mg/kg), acetaminophen (200 mg/kg) and ketorolac (3 mg/kg). In order to confirm that the effects of UCM765 and UCM924 were MT2 receptor-mediated, they were blocked by the selective MT2 receptor antagonist 4-Phenyl-2-propionamidotetralin (4P-PDOT; 10 mg/kg) injected 10 min prior the MT2 partial agonists. Results and discussion: UCM765 and UCM924 dose-dependently increased the temperature of the first hindpaw lick in the hot plate test (two-way repeated measure ANOVA: F3,20 = 23.57, P < 0.001 and F3,20 = 54.93, P < 0.001, respectively), and decreased the total time spent licking the injected hind paw in the formalin test (F4,24 = 11.32, P < 0.001 and F4,25 = 7.94, P < 0.001, respectively). Antinociceptive effects of UCM765 and UCM924 were maximal at the dose of 20 mg/kg. At this dose, the effects of UCM765 and UCM924 were similar to those produced by 200 mg/kg acetaminophen in the hot plate test, and by 3 mg/kg ketorolac and 150 mg/kg MLT in the formalin test. Only in the hot plate test, MLT (150 mg/kg) effects were greater than those of 20 mg/kg UCM765 (P=0.002) and UCM924 (P=0.002). However, UCM765 and UCM924 were collectively more pharmacologically potent than MLT since their dose necessary to reach an antinociceptive effect was 7.5-fold times lower than that of MLT. Notably, antinociceptive effects of the two MT2 partial agonists were blocked by the pretreatment with 4P-PDOT in both paradigms. These results demonstrate the analgesic properties of MT2 partial agonists in
acute/inflammatory pain models and confirm that the activation of MT2 receptor rather than of both MT1 and MT2 receptors is a novel target for analgesic drug development.


Poster

513. Inflammatory Pain

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 513.10/O41

Topic: D.08. Pain

Title: The role of activated peripheral kappa opioid receptors on the alleviation of arthritis pain

Authors: *S. O. MOON¹, H. HAN, male², E.-H. PARK, male², H. SUH, female²; ¹Col. of Med., Seoul, Korea, Republic of; ²korea university, seoul, Korea, Republic of

Abstract: For the treatment of pain, chronic and systemic opioid application can be used because of its strong analgesic effect but extensive activation of opioid receptors beyond the target can cause unwanted complications e.g. dysphoria, pruritis, respiratory depression and constipation. So the selective activation of peripheral opioid receptors of involved tissue can be considered as the better strategy to reduce pain without unwanted complications and to increase the effectiveness of analgesia by opioid. This study was done to clarify the effect of peripheral kappa opioid receptor activation on arthritic pain for the purpose of the possible use of peripheral opioid receptor to treat the arthritic pain. We investigated whether the activation of peripheral kappa opioid receptors (kORs) could reduce the nociceptive behavior and the response of sensitized primary mechanosensitive afferents (MSA) after carrageenan-induced arthritis in Sprague-Dawley rat. In order to activate peripheral kORs, we administered U50488 (selective agonist of kOR) into the knee joint. We evaluated the nociceptive behavior by using dynamic weight bearing test which can measure the decreased weight load on affected side during freely walking, and recorded MSA activity in knee joint using extracellular single nerve recording technique before and after the injection of U50488. Four hours after the induction of arthritis, the activation of peripheral kORs by U50488 (0.1nM, 10nM and 1µM) significantly recovered the decreased weight load onto the inflamed knee joint during freely walking compared to saline treatment. Consistent with these behavioral results, we also found that the intra-articular injection of U50488 (10nM and 1µM) significantly decreased the response of MSA activities to stimuli by von Frey filaments (6 and 26g) during 10 - 60 min after U50488 injection compared to saline treatment. However, in the absence of inflamed knee joint, the application of 1µM U50488 had
no effect on MSA activities. These results implicate that the activation of peripheral kOR in the knee joint can contribute to reducing the nociceptive behavior by decreasing the activity of MSA in inflamed knee joint.

Disclosures: S.O. Moon: None. H. Han: None. E. Park: None. H. Suh: None.

Poster

513. Inflammatory Pain

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

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Topic: D.08. Pain

Support: NIH-NIDCR #DE021888 (OJI)

Title: Role of trace metal-generated oxidative stress in Toll-like receptor 4 signaling (TLR-4) in synovial fibroblasts

Authors: *A. A. ALSOUSI, O. J. IGWE; Div. of Pharmacol. and Toxicology, Sch. of Pharm., Univ. of Missouri, Kansas City, MO

Abstract: Rheumatoid arthritis (RA) is a common chronic autoimmune systemic disease that affects the joints, especially in women. It is characterized by synovial inflammation, leading to tissue and bone destruction. It is proposed that RA is caused by a cross-talk between T and B lymphocytes, macrophages and synovial fibroblasts within the synovium. However, the importance of tissue reaction to immune stimulation, and not to the immune system itself, in causing the damage in RA, is considered a major cause of tissue damage. Reactive oxygen species (ROS) can interact with and modify cellular macromolecules, resulting in structural and/or functional changes in protein expression. High-mobility group box 1 (HMGB1), is a nuclear protein that can bind to TLR 4, and it is recognized as a representative damage activated molecular pattern (DAMP) molecule. HMGB1 is passively released from necrotic or stressed cells and is actively secreted by inflammatory cells, mediating the response to inflammation. We have recently demonstrated that oxidative stress can activate the TLR4 MyD88/NFκB-coupled pathway, causing release of both pro-inflammatory and anti-inflammatory cytokines. The exact molecular mechanism of these events is not clear. We will determine the role of ROS/TLR4-coupled activation as a regulator of biochemical crosstalk in synoviocytes. We used three trace metals, potassium peroxychromate (PPC), cuprous chloride (CuCl), and ferrous chloride FeCl₂ as an exogenous pro-oxidants and ROS generating systems to examine interactions between ROS and TLR4. Intracellular ROS (iROS) was quantified using immunofluorescence and flow
We used TLR4-signaling inhibitor CLI-095 and antioxidants to block ROS/TLR4-coupled interactions. HMGB1 release was measured upon pro-oxidants activation by western blot and ELISA. The trace metal ions (Cr\(^{3+}\), Cu\(^{+}\) and Fe\(^{2+}\)) increased iROS accumulation in a time-dependent manner. Generation of iROS was significantly decreased when Ebselen and EUK-134 free radical scavengers were applied. Pro-oxidants sensitized synoviocytes to increase the expression of TLR4. TLR4-signaling inhibitor CLI-095 abolished the increase in iROS. Pro-oxidants increased HMGB1 release from the nucleus to the cytoplasm. Our data show that trace metals as sources of iROS can generate oxidative stress. The oxidant-induced TLR4 activation can cause HMGB1 release, which may mediate proinflammatory actions and contribute to the pathogenesis of RA.

**Disclosures:** A.A. Alsousi: A. Employment/Salary (full or part-time); University of Missouri Kansas City. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; NIH-NIDCR #DE021888 (OJI). O.J. Igwe: None.

**Poster**

**513. Inflammatory Pain**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#: 513.12/O43**

**Topic:** D.08. Pain

**Support:** CB-2012/179294

**Title:** The alpha 5 subunit-containing GABAA receptors contribute to chronic pain

**Authors:** *M. BRAVO HERNANDEZ\(^1\), J. A. CORLETO\(^2\), P. BARRAGÁN-IGLESIAS\(^3\), R. GONZÁLEZ-RAMÍREZ\(^4\), J. B. PINEDA-FARIAS\(^1\), R. FELIX\(^3\), N. A. CALCUTT\(^3\), R. DELGADO-LEZAMA\(^5\), M. MARSALA\(^2,7\), V. GRANADOS-SOTO\(^1\);  
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**Abstract:** Chronic pain is characterized by a loss of pre- and post-synaptic inhibition and the occurrence of allodynia and hyperalgesia. After inflammation or nerve injury, GABAergic inputs...
are less inhibitory or even excitatory due to the disruption of the neuronal transmembrane chloride gradient by changes in the expression of Na+-K+-2Cl- co-transporter 1 (NKCC1) and/or K+-Cl- co-transporter 2 (KCC2). It has been recently proposed that alpha 5-subunit containing GABAA receptors (alpha 5-GABAA receptors) that mediate tonic inhibition might be involved in pain. The purpose of this study was to investigate the contribution of alpha 5-GABAA receptors in the loss of GABAergic inhibition and in formalin-induced long-lasting hypersensitivity. Formalin injection produced long-lasting secondary allodynia and hyperalgesia in both paws and impaired the rate-dependent depression of the Hofmann reflex (RDD) 6 days after injection. The peripheral and intrathecal pre-treatment or post-treatment with the alpha 5-GABAA receptor antagonist, L-655,708 (30-300 µM) prevented and reversed these long-lasting behaviors. Formalin injection increased alpha 5-GABAA receptors mRNA and protein expression in the spinal cord and dorsal root ganglia (DRG) at 3 days. alpha5-GABAA receptors were localized at the dorsal spinal cord, DRG and peripheral nerve fibers co-labeling with specific dorsal horn neurons and non-peptidergic fibers. Intrathecal administration of L-655,708 (300 µM) prevented and reversed the impairment of RDD. These results suggest that alpha 5-GABAA receptors play an important role in the loss of GABAergic inhibition and contribute to formalin-induced long-lasting secondary allodynia and hyperalgesia. Mariana Bravo-Hernández, Jorge B. Pineda-Farías and Paulino Barragán-Iglesias are Conacyt fellows. Ricardo González-Ramírez was supported by a post-doctoral fellowship from Conacyt (CB-2012/179294). Partially supported by Conacyt grants (CB-2012/179294 to VG-S, RF and RD-L).


Poster

513. Inflammatory Pain

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 513.13/O44

Topic: D.08. Pain

Support: CONACyT fellow grant 488726

CONACyT project 178027

Title: Participation of the potassium channels (K+) on the antinociceptive effect of peripheral administration of docosahexaenoico acid (DHA)
**Authors:** *A. Y. LANDA*¹², A. E. CHÁVEZ PIÑA²

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**Abstract:** Introduction: The adverse effects caused by the use of analgesics limit their use. Nociceptors, which are the first cells involved in pain, depend on the ion channels expressed in cells. Docosahexaenoico acid (DHA) is an omega-3 fatty acid, having an important role over pain regulation, through a generation of antinociceptive effect (analgesic effect); however, its mechanism is still not well-defined. Moreover, there is evidence of analgesic drugs (nonsteroidal anti-inflammatory NSAIDs and opioids), that produce its antinociceptive effect through the regulation of a numerous potassium channels (K⁺). For that reason, the purpose or this study was to demonstrate that the antinociceptive effect of local administration of DHA is generated by the activation of K⁺ channels.

**Material and methods:** female Wistar rats were administrated DHA (100 - 1778 µg/paw), 75 minutes before the formalin (1%); and percentage of antinociception was determined. In the same way, the antinociceptive mechanism of DHA was evaluated using specific potassium channels blockers for K ATP (glibenclamide and tolbutamide), Kv (4-aminoypyridine and tetraethylammonium), KCa-cl (iberiotoxin and caribdotoxin), KCa-cc (apamin and dequalinium) inhibition. Results: DHA administration generated antinociceptive effect in a dose dependent fashion. The best antinociceptive effect was achieved with 1000 µg/paw (70.05 ± 3.70 %) of DHA. The antinociceptive effect of DHA was reverted with the administration of the blockers of K ATP, KCa-cl and KCa-cl channels; but not with Kv.

**Conclusions:** DHA generates antinociceptive effect at peripheral level through the activation of potassium ATP-dependent, calcium large and small conductance K⁺ channels in the formalin test in the rat.

**Disclosures:** A.Y. Landa: None. A.E. Chávez Piña: None.
Title: SHANK3 regulates pain via possible peripheral and presynaptic mechanisms: Implication in pain dysregulation in autism

Authors: *Q. HAN*¹, Y. KIM¹, X. WANG², W. CHANG¹, Y. ZHANG¹, T. BERTA¹, F. TANG¹, Y.-H. JIANG¹,²,³, R.-R. JI¹,³; ¹Dept. of Anesthesiol., ²Dept. of Pediatrics, ³Dept. of Neurobio., Duke Univ. Med. Ctr., Durham, NC

Abstract: Individuals with autism spectrum disorders (ASDs) usually experience a wide range of sensory dysfunction related to touch, smell, taste, and pain. The self-injurious behaviors such as head banging, scratching, bruising, and biting in ASD patients could be explained as pain insensitivity. SHANK3 is a postsynaptic scaffold protein and has been implicated in ASDs. SHANK3 maps to the critical region of chromosome 22q13.3. Patients with 22q13 deletion syndrome (also known as Phelan-McDermid syndrome with deletion of entire SHANK3 gene) demonstrated high incidence (77%) of pain insensitivity. However, it is unclear if SHANK3 is expressed in primary sensory neurons in the peripheral nervous system and plays a role in regulating pain sensitivity. Using in situ hybridization and immunostaining, we found the expression of Shank3 mRNA and SHANK3 in primary sensory neurons of dorsal root ganglion (DRG) in mice. We also observed SHANK3 immunoreactivity (IR) in primary afferent terminals in the superficial spinal cord dorsal horn. SHANK3-IR in the spinal cord dorsal horn was markedly reduced after ablation of TRPV1-expressing primary afferents with resiniferatoxin (RTX), indicating that SHANK3 in DRG neurons can be transported to axonal terminals in the spinal dorsal horn and modulates nociceptive transmission via a presynaptic mechanism. We also test pain behaviors in newly generated full-length Shank3-/- mice with deletion of exons 4 to 22 (∆e4-22/-, referred as Shank3-/- mice). Shank3-/- mice displayed normal baseline pain. However, acute inflammatory pain in the formalin test and chronic inflammatory pain in the CFA model were markedly attenuated in Shank3-/- mice. Furthermore, knockdown of SHANK3 expression in DRG neurons with selective Shank3 siRNA, through peri-sciatic nerve injection, also reduced the formalin-induced inflammatory pain. Finally, SHANK3-IR was also found in human DRG neurons and nerve axons in the dorsal root. Our findings suggest that SHANK3 is required for the development of inflammatory pain via possible peripheral and presynaptic mechanism. Our results have also offered a mechanistic insight into pain deficits in autism patients.

Title: Local sympathetic denervation reduces pain behaviors and inflammation in the CFA model

Authors: S. CHEN, W. XIE, A. LI, J. A. STRONG, *J.-M. ZHANG;
Dept Anesthesiol, Univ. Cincinnati Coll Med., Cincinnati, OH

Abstract: Local sympathetic blockade is one treatment for some forms of chronic pain, and has recently been used as a pre-emptive analgesic for surgical pain. However, preclinical studies primarily use global sympathectomy or systemic blockade, or examine effects of sympathetic transmitters on immune cells and neurons in vitro. Both pro- and anti-inflammatory effects of the sympathetic nervous system have been reported in previous studies. In the study, we examined the effects of a very localized sympathectomy on the rat model of peripheral inflammation induced by injecting the hindpaw with Complete Freund’s Adjuvant (CFA). Localized sympathectomy was achieved by cutting the grey rami to the L4 and L5 sensory ganglia on one side. Anatomical studies indicate this should cause sympathetic denervation of the paw region but not of primary immune organs. Control animals received sham surgery. These surgeries had no effect on baseline pain behaviors. One to 2 weeks later, CFA (25 µL of 1 mg/ml of mycobacterium tuberculosis in paraffin oil diluted with 25 µL of buffered salt solution) was injected s.c. into the ipsilateral paw. The injection led to mechanical hypersensitivity and swelling of the hind paw that persisted up to 14 days (longest time examined). Mechanical pain behaviors (von Frey test, mechanical allodynia test) were reduced by prior local sympathectomy, at some points to as low as 30% of the values in sham operated animals, while paw swelling was reduced by 50 to 60%. Immunohistochemical examination of the paw tissue verified that sympathetic fibers were still absent 14 days after the CFA injection, and did not undergo compensatory sprouting from adjacent non-denervated regions. Density of CGRP-positive fibers was modestly reduced by the local sympathectomy. The results suggest that the sympathetic nerves have a relatively large, local, pro-inflammatory effect in this model.

Disclosures: S. Chen: None. W. Xie: None. A. Li: None. J.A. Strong: None. J. Zhang: None.
Title: Anti-cancer and analgesic effects of resolvin D-series in head and neck cancer

Authors: *Y. YE*, J. CURTIN, D. BERNABE, B. SCHMIDT


Abstract: Head and neck cancer patients have poor survival and experience excruciating pain. Cancer progression and pain result from shared pathways that involve chronic inflammation. Resolvin D-series are endogenous lipid mediators derived from omega-3 fatty-acid that exhibit potent anti-inflammatory actions. Based on this known mechanism we hypothesized that resolvin D-series (resolvin D1 and resolvin D2) can inhibit cancer growth and pain in head and neck cancer. We examined the effect of resolvin D1 and resolvin D2 on proliferation of a head and neck cancer cell (HSC-3) using a real-time cell analyzer (RTCA). To study the effect of resolvin D-series in vivo we produced a mouse oral cancer model by inoculating HSC-3 into the right hind paw of mice. We measured tumor size and nociceptive behaviors weekly for four weeks. Tumor size was measured using a plethysmometer. Mechanical sensitivity was tested with an electronic von Frey device and thermal sensitivity was measured using Hargreaves’ test. We found that both resolvin D1 and D2 reduced HSC-3 proliferation in a dose-dependent manner in vitro. 100ng Resolvin D1 treatment significantly increased paw withdrawal latency to thermal stimulation in cancer mice. 200ng resolvin D2 treatment significantly increased mechanical thresholds in cancer mice at week four compared to PBS treated cancer mice. Paw size was smaller in both resolvin D1 and resolvin D2 treated cancer mice, compared to PBS treated cancer mice. Our finding suggests resolvin D-series alleviate cancer pain and simultaneous reduces tumor burden. Ongoing studies are examining the specific immune cells and pro-inflammatory cytokines that resolvin D-series act on to exert its dual actions on both tumor progression and cancer pain.

Poster

513. Inflammatory Pain

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 513.17/O48

Topic: D.08. Pain

Support: IASP 2014 John J. Bonica Trainee Fellowship

Title: Characterization of the immune cell infiltrate in oral squamous carcinoma-induced cancer pain

Authors: *N. SCHEFF, B. L. SCHMIDT;
New York Univ., New York, NY

Abstract: Oral cancer patients report severe mechanically-induced pain. Opioids, despite their side effects and minimal efficacy, remain the primary therapeutic approach. Oral cancer pain is hypothesized to be due to tumor proliferation, perineural invasion, secretion of pro-nociceptive mediators and infiltration of the immune cells into the cancer microenvironment. Of those possible mechanisms immune infiltration into the cancer microenvironment is the least studied as non-steroidal anti-inflammatory drugs have minimal and unpredictable analgesic benefit. However, recent evidence shows that cytokines and chemokines produced by cancers can attract pro-inflammatory immune cells into tumors stimulating a proliferating microenvironment. We hypothesize that pro-nociceptive mediators secreted by human oral squamous cell carcinoma which include ET-1, NGF, ATP and proteases, not only sensitize and activate primary afferent neurons but also attract immune cells to the cancer microenvironment and cause pain. To simultaneously study the immune cell chemoattractant and pain-producing effects of cancer-secreted mediators, we injected oral squamous cell carcinoma (SCC) supernatant into the tongues of mice. Supernatant injection alone avoids the impact of tumor growth and perineural involvement. Immune cells from the tongue were typed and sorted with flow cytometry and pain behavior was measured with an objective operant assay (i.e., Dolognawmeter). Oral SCC supernatant exposure produced a roughly 60% increase in infiltration of total immune cells. Within this infiltrate, macrophages (CD11b+) population increased ~30% and T helper cell (CD3+ CD4+) population increased ~40%. Furthermore, a pro-inflammatory IL-17 producing CD4+ phenotype (Th17) was more prominent in tongues injected with oral SCC supernatant. This phenotype has been shown to induce production of pro-nociceptive cytokines, IL-6 and IL-8, as well as pro-angiogenic mediators and growth factors. Mice also displayed decreased orofacial function indicative of pain. We conclude that oral cancer pain is accompanied by an immune cell infiltrate which is triggered by cancer secreted products alone without tumor.
growth. Future studies will analyze the immune cell infiltrate accompanying oral carcinogenesis in a mouse oral cancer model that recapitulates the human condition.

Disclosures: N. Scheff: None. B.L. Schmidt: None.

Poster

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Topic: D.08. Pain

Support: CIHR

CFI

NSERC

Rita Allen Foundation/ American Pain Society

Title: Investigating the role of microglia and P2X7 receptors in monosodium iodoacetate induced joint pain

Authors: *M. J. MOUSSEAU*¹, A. PILAPIL², N. BURMA², J. MATYAS³, T. TRANG³; ¹Neurosci., ²Physiol. and Pharmacol., ³Univ. of Calgary, Calgary, AB, Canada

Abstract: Chronic joint pain is the cardinal symptom of all forms of arthritis, and is the underlying cause of disability in individuals afflicted by this disease. Although joint pathology is an underlying factor, some patients with severe joint pathology report little to no pain, while others with minor joint pathology experience debilitating and unremitting pain. This discordance between pain severity and peripheral pathology of the arthritic joint suggests the extent of joint damage is not necessarily a direct predictor of ensuing chronic pain. This study focused on the central mechanisms involved in modulating chronic osteoarthritis (OA) pain signaling. Emerging evidence suggests that altered pain processing within the spinal cord is a key determinant of joint pain. Here, we examined the involvement of spinal microglia and ATP-gated P2X7 receptors (P2X7R) in chronic OA pain. To induce OA pain, rats received intra-articular injection of monosodium iodoacetate (MIA), which resulted in a reduction in both mechanical and thermal nociceptive thresholds. The onset of mechanical allodynia and thermal hyperalgesia occurred as early as day 3 post-MIA injection, with alldynia persisting to least day 28. On day 7 post-MIA, we detected an increase in expression of microglial markers, Iba-1 and CD11b, in the ipsilateral
spinal dorsal horn. We assessed the OA joint pathology in rats 7 days post-MIA injection using the modified Mankin and OARSI scoring systems and found that MIA is sufficient to induce OA pathology. Given that P2X7Rs are involved in the pathophysiology of bone and cartilage disease, and are a locus through which microglia contribute to chronic pain, we asked whether P2X7Rs are causally involved in the development and expression of MIA-induced joint pain. We found that a 7-day intrathecal osmotic pump administration of the selective P2X7R antagonist A740003 attenuated the development of mechanical allodynia and thermal hyperalgesia in MIA-injected rats. In addition, we showed that an acute injection of A740003 in animals with established MIA-induced arthritis pain transiently reversed both the mechanical and thermal hypersensitivity. Collectively, our results demonstrate that spinal P2X7Rs are causally involved in the development and ongoing expression of arthritis pain.

**Disclosures:** M.J. Mousseau: None. A. Pilapil: None. N. Burma: None. J. Matyas: None. T. Trang: None.

**Poster**

513. Inflammatory Pain

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 513.19/P2

**Topic:** D.08. Pain

**Support:** NSERC

CIHR

CFI

**Title:** Potentiation of phase II formalin responses in zinc transporter-3 knockout mice

**Authors:** *C. Y. Fan*¹, B. B. McAllister², R. H. Dyck², T. Trang¹; ¹Neurosci., ²Psychology, Univ. of Calgary, Calgary, AB, Canada

**Abstract:** Zinc is abundant in the central nervous system (CNS), and its activity is implicated in neuropathic and inflammatory pain. Expression of zinc is high in synaptic vesicles, the localization of which is tightly regulated by the vesicular zinc transporter, ZnT-3. Vesicular zinc release into the synaptic cleft regulates pain transmission via inhibition of post-synaptic N-methyl-D-aspartate receptors. In this study, we found that ZnT-3 is also expressed in microglia, which are resident immune cells in the CNS. Under physiological conditions, microglia are in a surveillance state, with a small cell body and long processes that actively survey the...
environment. In the presence of damage and/or infection, microglia adopt a more activated state with a larger cell body, shorter processes, and an increase in the expression of cell surface markers alpha M integrin (cd11b) and ionized calcium-binding adapter molecule 1 (iba1). Aberrant microglia activation has been implicated in the development of neuropathic and inflammatory pain. Previous studies have shown the microglia activation can be triggered by extracellular zinc. In the present study, we asked whether ZnT-3 is critically involved in the sequelae of inflammatory pain. To test this, we examined the behavioural response of ZnT-3 knockout mice to a single intraplantar injection of formalin, which evoked a biphasic nociceptive response characterized by licking and shaking of the injected paw. We found that phase 1 of the formalin response (attributed to c-fibre activation) was comparable in ZnT-3 knockout and wild-type mice. In contrast, phase 2 nociceptive behaviours (associated with central sensitization) were significantly greater in the ZnT-3 knockout mice. We also determined that the deletion of ZnT-3 was associated with lower expression of the microglial and astrocytic markers, ionized calcium-binding adapter molecule 1 (iba1) and glial fibrillary acidic protein (GFAP), respectively. Collectively, our findings suggest that zinc transport mediated by ZnT-3 plays a critical role in the central sensitization associated with phase 2 of the formalin response.

surgical procedure with mid-thigh exposure of the sciatic nerve. Control animals underwent exposure only (n=12) and experimental animals underwent placement of littermate tail nucleus pulposus (n=12). Animals were evaluated throughout one week for mechanical allodynia by Von Frey testing, thermal hyperalgesia by heat withdrawal latency, cold allodynia by acetone testing, and gait stability by RotaRod testing. At sacrifice, immunohistochemistry was performed to identify perineural and intraneural macrophage and lymphocyte presence. Necessity of an inflammatory response was tested by attenuating systemic inflammation with intraperitoneal selenium delivery. Necessity of BDNF-dependent macrophage activity was tested using a tamoxifen-induced CreER BDNF knockout system. **Results:** Mice exposed to heterotopic NP stimulation demonstrated substantial mechanical allodynia, cold allodynia, and gait instability compared to controls. Intraneural macrophage infiltration was observed in this group, alongside associated autoreactive lymphocytes at the disc-nerve interface. Systemic selenium administration blocked this behavioral phenotype in the acute inflammatory phase. Knocking out inflammatory cell BDNF activity in the CreER animals eliminated macrophage migration and the painful phenotype. **Conclusion:** Non-compressive disc herniation leads to altered behavior in this animal disease model, with demonstrated need for intraneural macrophage migration. Strategies to decrease perineural inflammation or maintain integrity of the blood nerve barrier may be effective in treating painful disc-herniation radiculopathy. Understanding the pathophysiology of disc-herniation radiculopathy carries implications on effective non-surgical and intraoperative management of a highly prevalent condition. This may also help define the transition from acute inflammatory pain to chronic neuropathic pain

**Disclosures:** Y. Tu: None. M. Shamji: None. M. Salter: None.

**Poster**

1. Inflammatory Pain

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 513.21/P4

**Topic:** D.08. Pain

**Support:** DFG grant FKZ0316177A

**Title:** Cytokines of the IL-6/gp130 family increase the cAMP-ERK crosstalk in sensory neurons

**Authors:** *A. GARZA CARBAJAL, S. BROSIG, A. THIEL, T. HUCHO; Experimentelle Anästhesiologie und Schmerzforschung, Uniklinik Köln (aöR), Köln, Germany
Abstract: Sensory neurons in inflamed or damaged tissues are exposed to a multiplicity of extracellular factors. Beyond their direct effects on neurons, if these factors may also influence each other by modifying their intracellular signaling kinetics, is mostly unknown. Such mutual modulation may increase and/or prolong the signaling. Accordingly, such modulation would act as context detector and integrator for simultaneous or sequential stimuli. Sustained ERK activation in DRG neurons correlates with increased pain sensitivity. Additionally, increased or prolonged ERK activity downstream of cAMP production has been linked to pathological pain. Which factors and conditions govern this plasticity is mostly unknown. We now found that cytokines from the IL-6 family are able to modify the cAMP-ERK crosstalk in DRG neurons. Exposure to some members of this family increases the amplitude of cAMP induced ERK activity without modifying cAMP production or PKA activation. Cytokine effects on cAMP-ERK crosstalk are time and dose-dependent, require protein translation and were resistant to the wash out of the cytokine. The increment in the cAMP-ERK crosstalk was observed despite at which level the cAMP pathway was stimulated. Our results identify a cellular mechanism governing this plastic connectivity of the cAMP-ERK crosstalk. Such mechanism has potential relevance for the processes of nociceptor sensitization and neuronal regeneration.

Disclosures: A. Garza Carbajal: None. S. Brosig: None. A. Thiel: None. T. Hucho: None.

Poster

513. Inflammatory Pain

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Topic: D.08. Pain

Support: NS030045

DE017813

Title: F-actin links Epac-PKC signaling to sensitization of purinergic P2X3 receptors after inflammation

Authors: *Y. GU, C. WANG, G. LI, Y. CHEN, L.-Y. M. HUANG;
Univ. Texas Med. Br., Galveston, TX

Abstract: Sensitization of purinergic P2X3 receptors in dorsal root ganglion (DRG) neurons contributes to the production of the exaggerated nociceptive responses following inflammatory injury. Prostaglandin E2 (PGE2) produced in response to tissue injury has been found to
potentiate P2X3R-mediated responses in DRG neurons in both normal and complete Freund's adjuvant (CFA)-induced inflamed rats. In a previous study, we provided evidence that PGE2 potentiation of P2X3R responses depends only on PKA signaling in control neurons, but depends on both PKA and PKCε signaling in inflamed neurons. The activation of PKCε signaling, a result of PGE2-elicited activation of the cAMP-dependent guanine nucleotide exchange proteins (Epacs), gives rise to a much larger PGE2-induced enhancement after inflammation. It is not known, however, how the Epac-PKC signaling modulates P2X3R-mediated responses. Here we show that disruption of F-actin polymerization by either cytochalasin D (CD) or latrunculin A (LaA) blocks PGE2 enhancement of P2X3R-mediated ATP currents and inhibits alpha, beta_ATP-evoked nociceptive behaviors only under inflammatory conditions. The Epac activator, CPT, greatly increases the expression of F-actin. The increase is much reduced in the presence of PKC antagonists. Thus, Epac-PKC signaling modulates F-actin expression in DRGs. Furthermore, CPT also increases the membrane expression of P2X3Rs in DRG neurons and LaA greatly diminishes the CPT effect. These results suggest that F-actin is a critical link in the Epac-PKC signaling to bring about the sensitization P2X3Rs and the exaggerated pain responses following inflammation. (This work is supported by grants from National Institutes of Health).

Disclosures: Y. Gu: None. C. Wang: None. G. Li: None. Y. Chen: None. L.M. Huang: None.

Poster

513. Inflammatory Pain

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Topic: D.08. Pain

Support: Natural Science and Technology Research Council of Canada (RFN.356021)

Title: Preferred recycling pathway by internalized PGE2 EP4 receptor following agonist stimulation in cultured dorsal root ganglion neurons contributes to nociceptor sensitization

Authors: *W. MA*, B. ST-JACQUES;
1Psychiatry, McGill Univ., Verdun, QC, Canada; 2Douglas Mental Hlth. Univ. Inst., McGill Univ., Montreal, QC, Canada

Abstract: Chronic pain is an unmet clinical need which severely deteriorates the quality of life of individuals and imposes heavy financial burden on the society. It is generally believed that chronic pain results from sensitization of peripheral and central neurons along pain transmission pathway. Sensitization of nociceptive dorsal root ganglion (DRG) neurons (nociceptors) is a
prerequisite for transition from acute to chronic pain. Prostaglandin E2 (PGE2), a pain mediator over-produced in inflamed tissue, is known to sensitize nociceptors through its four EP receptors (EP1-4) expressed in DRG neurons. We recently showed that PGE2 or EP4 agonist stimulates EP4 externalization and this event was not only suppressed by the inhibitor of anterograde export, but also by the recycling inhibitor. These data suggest that EP4 recycling also contributes to agonist-enhanced EP4 surface abundance. In the current study, we tested this hypothesis by using antibody-feeding-based internalization and recycling assays as well as FITC-PGE2 binding assay. We observed that selective EP4 agonist 1-OH-PGE1 or CAY10850 time- and concentration-dependently increased EP4 internalization in cultured DRG neuron. Internalized EP4 receptors were predominantly localized in the early endosomes and recycling endosomes, but rarely in the lysosomes 1h after internalization, suggesting that internalized EP4 prefers to undergo the recycling pathway to return to cell surface than the degradation pathway to be degraded. These observations were also confirmed by FITC-PGE2 binding assay. We further revealed that 1-OH-PGE1 or CAY10850 time- and concentration-dependently increased EP4 recycling. Furthermore, double exposures to 1-OH-PGE1 significantly increased the levels of CGRP released from cultured DRG neurons compared to a single exposure or vehicle exposure. This event was blocked by the recycling inhibitor monencin pre-treated with the 1st EP4 agonist. Our data suggest that EP4 recycling contributes to agonist-induced cell surface trafficking and receptor sensitization. Facilitating EP4 externalization and recycling is a novel mechanism underlying PGE2-induced nociceptor sensitization.

Disclosures: W. Ma: None. B. St-Jacques: None.

Poster

513. Inflammatory Pain

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

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Topic: D.08. Pain

Support: FONDECYT #1151043

FONDECYT #1095089

ACT1114

Division of Intramural Research, NIDCR, NIH
Title: Targeted overexpression of Tumor Necrosis Factor-alpha increased Cyclin-dependent kinase 5 activity and the subsequent calcium influx in trigeminal ganglia neurons

Authors: *E. UTRERAS PURATICH*¹, P. ROZAS¹, P. LAZCANO¹, R. PIÑA², A. CHO³, A. TERSE¹, R. MADRID², C. GONZALEZ-BILLUALT¹, A. B. KULKARNI³;
¹Univ. of Chile. Fac. of Sci., Santiago, Chile; ²Univ. of Santiago, Santiago, Chile; ³NIDCR, Bethesda, MD

Abstract: We reported earlier that TNF-alpha, a proinflammatory cytokine implicated in many inflammatory disorders that cause orofacial pain, increases kinase activity of Cdk5, a key kinase involved in brain development and function, and pain signaling. To investigate a potential mechanism underlying inflammation in trigeminal ganglia (TG), we engineered a transgenic mouse model (TNFglo) that could conditionally overexpresses TNF-alpha upon genomic recombination by Cre recombinase. TNFglo mice were bred with Nav1.8-Cre mice that express the Cre recombinase predominantly in the sensory neurons. TNFglo; Nav1.8-Cre (cTg TNF) mice appeared normal without any gross phenotype. However, these mice displayed significant increase in TNF-alpha levels causing activation of NF-kappaB signaling pathway in TG. Additionally, increased IL-6 and MCP-1 levels along with intense immunostaining for Iba1 and GFAP indicated activation of microglia and astrocytes in TG. Most importantly, cTg TNF mice displayed increased p35 protein level and Cdk5 kinase activity in TG, and this increase was associated with elevated phospho-T407-TRPV1 and Ca+2 influx. Interestingly, this effect was reversed by roscovitine, a specific inhibitor of Cdk5, suggesting that TNF-alpha overexpression caused sensitization of TRPV1 channel, which is partially mediated by Cdk5. In summary, cTg TNF mouse model would be valuable in defining new approaches to investigate orofacial pain mechanism involving TNF-alpha mediated pain signaling.


Poster
513. Inflammatory Pain

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 513.25/P8

Topic: D.08. Pain

Support: National Science Foundation of China #81271239 (CM)
Title: The role of neuronal Fc-gamma receptor I in antigen-specific pain

Authors: H. JIANG, X. SHEN, Z. CHEN, F. LIU, T. WANG, B. YUAN, Y. XIE, *C. MA; Inst. of Basic Med. Sciences, CAMS&PUMC, Beijing, China

Abstract: Antigen-specific immune disorders are often accompanied by pain but the underlying mechanisms are not fully understood. In previous studies we found that Fc-gamma-Receptors (including FcγRI, IIb and III) were expressed in dorsal root ganglion (DRG) neurons in rats and can be specifically activated by IgG immune complex (IgG-IC). Intradermal injection of IgG-IC to the rat hindpaw induced both mechanical and thermal hyperalgesia. This effect can be reduced by a high concentration of nonspecific IgG but not by mast cell stabilizer or histamine receptor blocker, indicating a role of FcγRI in pain transmission. Calcium imaging revealed that IgG-IC induced intracellular calcium increase in small-diameter, but not in the medium- or large-diameter dissociated DRG neurons. Electrophysiology recordings, both in vitro and in vivo, demonstrated that IgG-IC induced hyperexcitability and action potential discharges only in a subpopulation of C-nociceptive DRG neurons. The high-affinity activating IgG-Fc receptor FcγRI (CD64) were found upregulated in the relevant DRGs in a rat model of antigen-induced arthritis, with an increased incidence of spontaneous activities and enhanced responses to IgG-IC in nociceptive DRG neurons in vivo. These results suggest that nociceptive neuronal FcγRI may contribute to painful conditions induced by IgG-IC in antigen-specific immune disorders.


Poster
513. Inflammatory Pain

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 513.26/P9

Topic: D.08. Pain

Support: DFG Grant LE3210/2

Title: The cellular and molecular identity of “silent” nociceptors
**Authors:** *S. G. LECHNER, A. ARCOURT, V. PRATO;
Heidelberg Univ., Heidelberg, Germany

**Abstract:** In addition to mechano-nociceptors and polymodal nociceptors, C-fibres and A-delta-fibers also comprise a significant proportion of fibers that are insensitive to mechanical stimuli under normal conditions, but become sensitized to such stimuli during inflammation. These so-called “silent” nociceptors have been found in many species, including mice, and were shown to innervate the viscera, deep somatic tissues and the skin. Here we show that silent nociceptors are characterized by the expression of the alpha-3 subunit of the nicotinic acetylcholine receptor (Chrna3) and that they can be “un-silenced” by NGF induced upregulation of mechanically-gated ion channels. Chrna3+ neurons account for app. 8% of all DRG neurons, they express the NGF receptor trkA, but do not express NF200 or bind the non-peptidergic neuron marker Isolectin B4. Moreover, Chrna3+ neurons are enriched in thoracic and sacral DRGs and are only present in small numbers in cervical and lumbar DRGs. Consistent with this, Chrna3+ afferents are present in large numbers in the viscera and in deep somatic tissues but are rather rare in the skin, which was reminiscent of the previously described distribution of mechanically-insensitive silent nociceptors. Patch-clamp recordings from cultured DRG neurons further showed that Chrna3+ neurons fire action potentials that exhibit a nociceptor-specific hump on the falling phase and that they are indeed insensitive to mechanical stimuli. However, following treatment with NGF, which is known to “un-silence” silent nociceptors, all Chrna3+ neurons acquired mechanosensitivity. The NGF-induced acquisition of mechanosensitivity is mediated by the MAP-Kinase pathway and requires gene transcription. Taken together, our results suggest that Chrna3+ neurons represent a functionally unique subpopulation of peptidergic c-fiber nociceptors that shares many features with silent nociceptors. To directly test this hypothesis, we analyzed the functional properties of Chrna3+ afferents in the intact skin using single unit teased fiber recordings from a skin-nerve preparation. To allow the unequivocal identification of Chrna3+ afferents in these experiments, we generated mice in which Channelrhodopsin-2 (ChR2) expression is confined to Chrna3+ afferents. Consistent with the results from our immunocytochemical studies and patch clamp recordings, transdermal illumination with blue light only evoked action potentials in slowly conducting unmyelinated c-fibers that were insensitive to mechanical stimulation of the skin, demonstrating that Chrna3+ neurons are indeed mechanically insensitive silent nociceptors.

**Disclosures:** S.G. Lechner: None. A. Arcourt: None. V. Prato: None.

**Poster**

513. Inflammatory Pain

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM
Elucidating the role of anti-inflammatory cytokine interleukin-10 in peripheral neuropathic pain

Authors: *A. G. VANDERWALL*¹,², S. NOOR¹, N. W. HARRIS¹, J. J. SANCHEZ¹, M. S. SUN¹, R. A. WHITEHEAD¹,², X. O. YANG¹, E. D. MILLIGAN¹;²;¹Neurosciences, ²Anesthesiol. and Critical Care, ³Mol. Genet. and Microbiology, Univ. of New Mexico Sch. of Med., Albuquerque, NM

Abstract: Current treatments for neuropathic pain primarily target neurons, and are often ineffective for patients. Spinal glia have been implicated in neuropathic pain such as mechanical allodynia (pathological sensitivity to light touch). Glial pro-inflammatory cytokines are both necessary and sufficient for the development of chronic neuropathic pain demonstrated in a variety of animal models. Interleukin-10 (IL-10) is a powerful endogenous anti-inflammatory cytokine. Intrathecal (i.t., peri-spinal) viral vectors encoding IL-10 transiently suppress peripheral neuropathic pain for several weeks. Surprisingly, i.t. application of non-viral IL-10 transgene dramatically improves the duration of pain suppression, lasting 90+ days. Despite numerous reports demonstrating prolonged non-viral spinal IL-10 efficacy in pain suppression, the underlying mechanisms remain poorly understood. Furthermore, given endogenous IL-10 can impact transgene uptake and also the development and progression of neuropathic pain itself, we first investigated whether IL-10 deficiency alters the initiation and maintenance of allodynia caused by chronic constriction injury (CCI) of the sciatic nerve. Subsequent to characterizing the behavioral phenotype in IL-10 knockout (KO) mice following CCI, we then sought to determine whether i.t. IL-10 could alter the magnitude and duration of allodynia. In behaviorally verified mice, we aimed to quantify the spinal IL-10 protein expression timecourse in specific cell types using IL-10 knockout (KO) mice under chronic neuropathic conditions. Baseline (BL) hindpaw response thresholds to light touch stimuli (von Frey fiber test) of adult male IL-10 KO or C57BL/6 wild type (WT) mice were assessed prior to CCI or sham surgery. Thresholds were reassessed 1, 3, 5, 7, and 8 days post-surgery, then every 4-5 days until allodynia resolved. IL-10 KO mice exhibit exaggerated allodynia within 24-hours post-surgery compared to WT mice, but with comparable levels at subsequent timepoints, with allodynia resolved by Day 42. While
currently ongoing, pilot data demonstrates that a single i.t. co-injection (10.5μL total) of non-viral plasmid DNA encoding the IL-10 transgene (3μg) with the adjuvant D-mannose (25μg) on day 5 post-surgery produced reversal from allodynia to BL levels within 2 days in both IL-10 KO and WT mice. Ongoing studies continue to monitor hindpaw thresholds every 3-4 days until Day 42 post-injection. Future studies aim to identify IL-10 transgene expression in discrete spinal, spleen & lymph node cell populations. Other phenotypic markers of immune-cell activation will be analyzed using FLOW cytometric and ELISA techniques.


Poster
513. Inflammatory Pain

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Title: MicroRNA-219 in ventral tegmental area regulates pain by targeting CC2D1A

Jiangsu Province Key Lab. of Anesthesiol., Xuzhou Med. Col., Jiangsu, China

Abstract: Chronic pain is still a basic science and clinical challenge. Unraveling of the neurobiological mechanisms involved in chronic pain will offer novel targets for the development of therapeutic strategies. Recent studies have showed that maladaptation of ventral tegmental area (VTA) dopaminergic neurons is implicated in pain modulation. A growing body of evidence has also demonstrated that epigenetic mechanisms participated in maladaptation of VTA dopaminergic neurons in the different pathological status like depression and drug addiction. Here, we demonstrated that microRNA-219 (miR-219) in the VTA regulates pain by targeting CC2D1A. We found that the expression level of miR-219 in VTA decreased after
intraplantar injection of CFA. Over-expression of miR-219 attenuated CFA-induced thermal hypersensitivity; while, down-regulation of miR-219 dramatically decreased the paw withdrawal latency. Double staining showed that the GFP-tag of miR-219 was colocalized with glial fibrillary acidic protein (GFAP), but not with TH-positive DA neurons. Intra-VTA injection of fluorocitrate partially reversed the miR-219 down-regulation-induced thermal hyperalgesia. Down-regulation of miR-219 also increased the levels of proinflammatory cytokines in VTA and induced dopaminergic neurons activation. MiR-219 could directly regulate the expression of CC2D1A in vivo and in vitro. Genetic disruption of CC2D1A/NF-κB pathway by siRNA could reverse the miR-219 down-regulation-induced thermal hyperalgesia, increased levels of proinflammatory cytokines and dopaminergic neurons activation. Furthermore, inhibition of VTA DA neurons by microinjection of baclofen into VTA reversed the miR-219 down-regulation induced pain hyperalgesia. Collectively, our results support an integral role for VTA miR-219 in pain modulation by targeting CC2D1A and might therefore provide a novel target for pain therapy.


Poster
513. Inflammatory Pain

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 513.29/P12

Topic: D.08. Pain

Support: NIH Grant R01DA006736

IASP Grant John J. Bonica Fellowship

Title: Protein and transcript levels for Gα proteins in the rostral ventromedial medulla and dorsal horn of the spinal cord of rats with peripheral inflammatory injury

Authors: *A.-S. WATTIEZ, C. M. SANDE, R. Y. WALDER, D. L. HAMMOND; Anesthesia Res., Univ. of Iowa, Iowa City, IA

Abstract: Inflammation leads to persistent changes in the pharmacology and physiology of rostral ventromedial medulla (RVM) neurons, inducing a time-dependent increase in the potency of Mu-opioid receptor (MOPr) agonists. The mechanism responsible for this enhancement has not yet been fully discerned. We hypothesize that under conditions of peripheral inflammatory
pain the subcellular pathways to which the MOPr couples may change. Possibilities include a decrement in Ga or Ga proteins, or an increase in coupling to Ga or Ga proteins. This hypothesis led us to investigate the protein and transcript levels of the different Ga proteins subtypes in the RVM and the spinal cord - first relay of the pain pathway that can be modulated by neurons projecting from the RVM - of animals with or without persistent inflammatory pain. These experiments determined the protein and transcript levels of Ga, Ga, Ga, Ga, and Ga in the RVM and dorsal horn of the spinal cord of rats following ipl injection of complete Freund's adjuvant (CFA) or saline in the hind paw. Four days or two weeks after ipl injection, tissue punches were obtained from the RVM and the ipsilateral dorsal horn of the spinal cord. Transcript levels were determined by RT-qPCR experiments and were normalized to levels of β-actin transcripts in the same sample. The amounts of Ga protein in the different conditions were assessed by Western blot experiments, and normalized to total protein staining in the same sample. Transcript levels for Ga protein subtypes were similar in between tissue and time points. Ga transcripts were the most abundant, followed by Ga and Ga. Ga was half as abundant as Ga, and the 2 least abundant transcripts were Ga and Ga. Surprisingly, the inflammation induced by ipl injection of CFA did not change Ga transcript levels for any of the subtypes in either the RVM or the dorsal horn. The finding that levels of Ga transcript were higher than Ga or Ga subunits in the RVM and the spinal cord was unexpected, and merits further study. At the protein level, preliminary Western blot data suggest that ipl injection of CFA decreases the amount of Ga and Ga protein in the dorsal horn both 4 days and 2 weeks after the injection. Experiments are underway to determine how persistent inflammatory nociception affects the abundance of the other Ga protein subtypes both in the dorsal horn and the RVM. These results provide suggestive evidence that persistent inflammatory injury may fundamentally change the Ga protein landscape in the RVM and the dorsal horn of the spinal cord, two major sites of pain transmission and modulation.


Poster

513. Inflammatory Pain

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 513.30/P13

Topic: D.08. Pain

Support: NIH Grant NS45594
High fat diet increases pain behaviors in a rat model of low back pain

Authors: *J. A. STRONG*¹, W. XIE¹, M. PRINT¹, Y. M. ULTRICH-LAI², J.-M. ZHANG¹;
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Abstract: Obesity is associated with a chronic low-grade inflammation and activation of components of the sympathetic nervous system. Human studies indicate that some chronic pain conditions are more prevalent in obese patients. These conditions include low back pain, and evidence suggests this is not simply explained by the muscles being subjected to increased weight bearing. We used our established model of low back pain to investigate interactions between high fat diets and pain. In this model, longstanding mechanical pain behaviors are induced by locally inflaming the L5 dorsal root ganglion with the immune stimulator zymosan in CFA (LID model). Young adult male rats were ad libitum-fed either high fat diet (HFD; 40% of calories from fat, primarily butter fat) or conventional low fat chow. Blood samples were obtained every 2 weeks for measurement of plasma adipokines and inflammatory cytokines. HFD had no effect on baseline mechanical sensitivity. The LID model was implemented after 6 weeks on the diet and the diet was continued for the rest of the experiment. The amount of zymosan injected was reduced to 1/10 of the amount usually used for this model, with the result that animals on normal chow displayed no increased mechanical pain sensitivity. However, animals on HFD showed marked mechanical hypersensitivity (von Frey test) and mechanical allodynia (responses to light strokes), similar to that previously seen at the higher dose of zymosan in rats on conventional low fat chow diets. Cold responding (acetone test) was also elevated by HFD on some days. Similar behavioral results were obtained in male and female rats. The marked effects of HFD on pain behaviors in this model should facilitate investigation of mechanisms for interactions between dietary fat and pain.


Poster

514. Opioids and other Analgesics

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 514.01/P14

Topic: D.08. Pain

Support: CIHR Operating Grant
Title: Attenuation of opioid analgesia in T-cell deficient mice

Authors: *S. F. ROSEN, I. WALTERS, S. SOTOCINAL, J. S. MOGIL;
Psychology, McGill Univ., Montreal, QC, Canada

Abstract: It is now known that neurons are not the only cell type involved in pain processing, which involves Schwann cells, satellite cells, and cells of the immune system, such as microglia, macrophages, and T-cells. Many pain researchers have adopted the use of T-cell deficient mice in their experimental methods to elucidate the role of T-cells in neuropathic pain (Fitzgerald et al., 2009; Zuo et al., 2013), and T cells have been shown to release endogenous opioids (Dietrich et al., 2011). While it is well known that opioids have varying effects on the immune system, very little attention has been given to how the immune system may affect opioid regulation. We now have evidence that T-cell deficient mice (CD-1 nude and Rag1 null mutant) exhibit pronounced deficiencies in morphine analgesia, measured using the tail withdrawal or formalin test. We also observe a sex difference in morphine analgesia in the nude mice, which appears to be dose dependent; female nude mice need a much higher dose of morphine to exhibit analgesia than male nude mice. Furthermore, T-cell deficient mice do not exhibit stress-induced analgesia after restraint. These results suggest that T-cells play a role in opioid-mediated analgesia, in a sex dependent manner. Current experiments are investigating the mechanism behind this phenomenon.

Disclosures: S.F. Rosen: None. I. Walters: None. S. sotocinal: None. J.S. Mogil: None.

Poster

514. Opioids and other Analgesics

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 514.02/P15

Topic: D.08. Pain

Support: 1R01DA035931 from NIDA to CAF

The College of Pharmacy, Noble Award

T32DA0070970 supported the training of CDP and CCC

Title: AAV-mediated human arginine decarboxylase (hADC) overexpression modulates opioid tolerance and reinstatement of opioid self-administration
Abstract: Background: Agmatine, decarboxylated L-arginine, has been shown to reduce opioid analgesic tolerance and opioid-induced drug addiction. Agmatine inhibits NMDA receptor dependent-behavior and spinal long term potentiation; the mechanism by which agmatine reduces pathological neuroadaptation likely involves that system. Since agmatine is endogenous, it may be possible to increase local agmatine levels by overexpression of its enzyme (arginine decarboxylase, ADC). We have developed an adeno-associated viral vector (AAV) to express human ADC in peripheral neurons and the CNS. We then compared development of intrathecal endomorphin-2 induced analgesic tolerance in subjects treated with vector (AAV-hADC) or saline. We also evaluated the impact of AAV9-hADC delivered to the nucleus accumbens in a model of opioid reinstatement. Methods: Endo-2 tolerance. Mice were pre-treated with either AAV5-hADC or saline. Endo-2 tolerance was induced acutely with a 10 nmol intrathecal injection of Endo-2 or saline. Cumulative dose-response curves to Endo-2 were constructed in each of the groups (Saline-Saline, Saline-Endo-2, AAV5-hADC-saline, AAV5-hADC-Endo-2.) The impact of immunoneutralization of putative endogenously produced agmatine was assessed through the intrathecal pre-treatment of anti-agmatine IgG versus normal IgG. After testing, spinal cords were tested via HPLC analysis for agmatine content. Oxycodone Reinstatement. Mice were trained to lever press for oral oxycodone. Following abstinence, mice were injected with either AAV9-hADC or saline bilaterally in the nucleus accumbens. Reinstatement to oral oxycodone was assessed at 3 weeks post-injection. Results: A rightward shift in the Endo-2 dose-response curve was observed in control subjects. However, in subjects pre-treated with AAV5-hADC, the ED50 values were equivalent between the Endo-2 and saline groups, showing lack of tolerance. In AAV5-hADC subjects pre-treated with anti-Ag IgG (but not normal IgG) Endo-2 tolerance developed, suggesting the importance of endogenous agmatine. Spinal levels of agmatine were elevated in the AAV5-hADC-treated subjects versus controls. Also, subjects treated with AAV9-hADC demonstrated a trend toward reduced reinstatement to oxycodone relative to those treated with saline. Conclusion: We interpret the observed inhibition of endomorphin-2 analgesic tolerance in the AAV5-hADC treatment, which was reversed by the pre-treatment of the anti-agmatine IgG, as attributable to agmatine produced from the overexpression of hADC. This is supported by the elevation in agmatine spinal cord levels in the hADC-treated subjects.

**514. Opioids and other Analgesics**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 514.03/P16

**Topic:** D.08. Pain

**Title:** CaMKIIα controls the biogenesis of let-7 microRNAs in opioid tolerance

**Authors:** *Y. HE, Z. J. WANG; Biopharmaceutical Sci., Univ. of Illinois at Chicago, Chicago, IL

**Abstract:** Emerging evidence suggests that microRNAs (miRNAs) - mediated cellular adaptations are critical for drug addiction. We previously reported that let-7 family miRNAs contribute to the development of opioid tolerance by targeting the µ opioid receptor. Chronic morphine treatment induced a marked increase of let-7 expression, which functionally correlated with the development of opioid tolerance. The aim of this study was to understand the mechanisms how let-7 is regulated by opioids. We first determined the transcription status of let-7 and found that the expression of primary let-7 (pri-let-7) remained unchanged in SH-SY5Y cells that were treated with morphine (1 µM, for 48 h). In agreement with the in vitro observation, chronic morphine tolerance (one 75 mg morphine pellet/mouse, s.c.) did not alter the level of pri-let-7 in mouse brain front cortex region. These findings suggested that the robust elevation of let-7 occurred at the post-transcriptional level. In the presence of KN93, inhibitor of Ca²⁺/calmodulin-dependent protein kinase II (CaMKII), chronic morphine treatment failed to generate let-7 up-regulation in SH-SY5Y cells. We further determined whether inactivation of CaMKIIα by T286A point mutation would affect let-7 expression and opioid tolerance. Indeed, antinociceptive tolerance was absent in CaMKIIα<sub>T286A</sub> mutant mice. Meanwhile, the level of let-7 in CaMKIIα<sub>T286A</sub> mutant mice was much lower than that in wild-type mice, and was resistant to chronic morphine stimulation. Taken together, these data suggested that the activity of CaMKIIα was essential for the biogenesis of let-7 in opioid tolerance.

**Disclosures:** Y. He: None. Z.J. Wang: None.

**Poster**

**514. Opioids and other Analgesics**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 514.04/P17
Title: Study of neuropeptides involved in opioid induced hyperalgesia through liquid chromatography mass spectrometry

Abstract: Opioid induced hyperalgesia (OIH) refers to a paradoxical nociceptive sensitization state caused by exposure to opioids which are used for the treatment of chronic pain. Although the precise molecular mechanism of OIH has not been fully characterized, changes in neuropeptides within the pain pathway are hypothesized to lead in OIH. The role of specific neuropeptides (eg. dynorphin) have been suggested, but there has not been a comprehensive peptidomics study of nervous system regions involved in OIH. To better understand the role of neuropeptides in OIH, we characterized the endogenous peptides in several critical brain regions involved in OIH using a rodent model. OIH was established by injecting escalating doses of morphine twice daily for 4 days. Four different defined regions in the OIH biological circuits were isolated from rats, including the periaqueductal grey, nucleus accumbens, rostroventral medulla and dorsal horn of the spinal cord. Peptides were extracted from tissues and analyzed by nanoLC coupled to a high resolution Q-TOF mass spectrometer. PEAKS software was employed to perform database searches for peptide identification. Initially 43 neuropeptides derived from 11 prohormones have been identified. Opioid peptides play important roles in pain by interacting with opioid receptors. 12 opioid peptides were identified and pro-enkephalin derived peptides were detected in all the regions investigated. Other pain-related peptides were also detected, including substance P, short neuropeptide K and somatostatin (77-87) and pituitary adenylate cyclase-activating polypeptide (111-128). We also observed cerebellin in spinal cord which affects synapse plasticity, and may regulate the neural adaptations result in OIH. In addition, CGRP (19-37) was detected in the dorsal horn. This truncated CGRP has an arginine at the preceding position, which suggests it may not be a degradation product but a peptide enzymatically processed at the monobasic basic site. This work has laid the groundwork for exploring the role of neuropeptides in OIH. In the future, a comparative peptide quantitation study will be conducted between OIH and control groups to discover signaling peptides whose expressions are significantly influenced by OIH. Physiological experiments on the molecular level will help discover their functions and lead to a better understanding of the mechanism of this prevalent disease.

Title: Delta opioid receptor functional competence is inhibited by lipoxygenase metabolites in the carrageenan model of inflammatory pain

Authors: L. C. SULLIVAN1,*W. P. CLARKE2, K. A. BERG1;
1Pharmacol., 2Univ. Texas Hlth. Sci. Ctr., San Antonio, TX

Abstract: The function of delta opioid receptors (DOR) expressed by peripheral pain-sensing neurons (nociceptors) is regulated by both cyclooxygenase- (COX) and lipoxygenase (LOX)-dependent arachidonic acid (AA) metabolites. Unlike opioid receptors expressed in other areas (e.g. CNS) or in heterologous receptor systems, opioid receptors expressed by nociceptors are functionally inactive with respect to producing antinociception or inhibition of adenylyl cyclase activity. However, following brief exposure to inflammatory mediators such as bradykinin (BK) or AA, opioid receptors become responsive to opioid agonists (functionally competent) due to the actions of a COX-dependent metabolite of AA. Moreover, in response to metabolism of AA by LOX, DOR returns to an unresponsive state that is refractory to re-induction of functional competence (Sullivan et al., 2015, J Pharmacol Exp Ther 353(1):44-51). Here we show that DOR functional competence can also be induced in vivo by brief exposure to carrageenan.

Carrageenan produces a state of inflammation that involves a variety of inflammatory mediators, including BK and AA. Intraplantar (i.pl.) injection of carrageenan (500 µg) produced thermal allodynia that lasted for at least 24 h. When tested 15 min after carrageenan, injection of the DOR agonist, DPDPE (20 µg, i.pl.) blocked carrageenan-induced thermal allodynia, indicating that DOR was functionally competent. When tested 3 h or 24 h after carrageenan, DPDPE did not inhibit thermal allodynia indicating that DOR had become unresponsive. However, following injection (i.pl.) of the 12- and 15-LOX inhibitors, Luteolin (3 ug) and Baicalein (3 ug), responsiveness to DPDPE (DOR functional competence) at the 3 h and 24 h time points was restored. These data indicate that opioid receptor system functional competence induced in response to local inflammation produced by carrageenan is transient. However, similar to our
findings with AA-mediated induction of DOR functional competence, the duration of functional competence can be increased by inhibition of LOX, suggesting that a LOX-dependent AA metabolite produces an unresponsive state of DOR. Results of this study further underscore the extraordinary regulation of the function of opioid receptors expressed by peripheral sensory neurons. Peripherally-restricted opioids may be very effective at treating pain due to inflammation.


Poster

514. Opioids and other Analgesics

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 514.06/P19

Topic: D.08. Pain

Support: DA037572

DA024865

GM106035

COSTAR 2T32DE014318-12A

Title: 6’-Guanidinonaltrindole (6’-GNTI) targets DOR-KOR heteromers in peripheral sensory neurons

Authors: *B. A. MCGUIRE, W. P. CLARKE, K. A. BERG;
UT Hlth. Sci. Ctr., San Antonio, TX

Abstract: Originally developed as a KOR agonist, 6’-GNTI has been shown to have agonist activity at KOR for Gi-protein-mediated signaling in brain and HEK cells and affinity (but no efficacy) for DOR. However, in peripheral nociceptors, we have found that 6’-GNTI agonist activity for Gi-mediated responses requires expression of both KOR and DOR. In primary cultures of peripheral sensory neurons, 6’-GNTI inhibited adenylyl cyclase activity by 65%. However, following siRNA knockdown of DOR, 6’-GNTI had no effect on adenylyl cyclase activity but antagonized the response to the KOR agonist, U50488. Similarly, when KOR expression was reduced with siRNA treatment, 6’-GNTI had no effect on adenylyl cyclase activity, but antagonized the response to the DOR agonist, DPDPE. Thus, 6’-GNTI has affinity, but not efficacy (i.e. acts as an antagonist) when either DOR or KOR expression is reduced.
Importantly, these data suggest that 6'-GNTI efficacy requires activation of DOR-KOR heteromers in peripheral nociceptors. Because we have demonstrated that allosteric interactions between protomers of DOR-KOR heteromers can regulate DOR and KOR agonist potency and efficacy in peripheral nociceptors, (Berg et al., 2012, Mol Pharmacol 81:264-272; and Jacobs et al this meeting), we next tested the hypothesis that 6'-GNTI occupancy of the DOR protomer of DOR-KOR heteromers allosterically enhances its own efficacy at KOR. In peripheral sensory neuron cultures, we measured 6'-GNTI-mediated responses in the presence of several different selective DOR antagonists. Both inhibition of adenylyl cyclase activity as well as antinociception in response to 6'-GNTI were reduced in the presence of the DOR antagonists naltrindole (2nM, 100x Ki) or 7-Benzlidenealtrexone (1nM, 100x Ki). By contrast, naltriben (NTB, 1nM, 100x Ki), fully substituted for 6'-GNTI occupancy of DOR. The concentration response curves of 6'-GNTI for inhibition of adenylyl cyclase activity were superimposable in the absence (DOR occupancy by 6'-GNTI) and presence of NTB (DOR occupancy by NTB). Similarly, in a behavioral model of thermal allodynia, 6'-GNTI produced the same robust antinociceptive response in the presence or absence of NTB. These data are consistent with the hypothesis that 6'-GNTI occupancy of DOR augments its own efficacy at KOR through allosteric interactions between DOR and KOR within the DOR-KOR heteromer.


Poster

514. Opioids and other Analgesics

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

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Topic: D.08. Pain

Support: NIH Grant GM106035

NIH Grant DA038645

William and Ella Owens Medical Research Foundation

Title: Prolonged functional competence of delta opioid-kappa opioid receptor (DOR-KOR) heteromers in the rat carrageenan model of inflammatory pain

Abstract: Opioid receptor systems expressed by peripheral pain-sensing neurons (nociceptors) are under dual regulatory control by cyclooxygenase (COX) and lipoxygenase (LOX) dependent arachidonic acid (AA) metabolites. For example, delta opioid receptors (DOR) are functionally inactive under basal conditions, but become responsive (i.e., functionally competent) following exposure to inflammatory mediators (e.g., carrageenan, bradykinin (BK) or AA) that produce COX-dependent AA metabolites. Following induction of functional competence by AA, DOR reverts to a basal non-responsive state that is refractory to re-induction of functional competence. This refractory, non-responsive state can be blocked by inhibiting LOX thereby allowing DOR functional competence to be re-induced (Sullivan et al., 2015, J Pharmacol Exp Ther 353: 44-51). Recently we reported that, in peripheral nociceptors, DOR forms heteromers with kappa opioid receptors (KOR) that produce robust antinociceptive responses following exposure to BK (Berg et al., 2012, Mol Pharmacol 54:94-104). Here we sought to explore DOR-KOR heteromer responsiveness in the carrageenan model of inflammatory pain. We examined the actions of the DOR agonist, DPDPE, the KOR agonist, U50488 and the DOR-KOR heteromer agonist, 6'-GNTI, to inhibit carrageenan induced thermal allodynia in the rat. When tested 15 min after intraplantar (i.pl) injection of carrageenan (500 ug), all agonists were effective at reducing carrageenan-induced thermal allodynia. When tested either at 3h or 24h post-injection of carrageenan, neither DPDPE nor U50488 reduced the thermal allodynia. However, responsiveness (i.e. functional competence) was restored by inhibition of LOX. Interestingly and by contrast, 6'-GNTI remained capable of inhibiting carrageenan-induced thermal allodynia for up to 24h (longest period tested) post-injection. These data suggest that DOR-KOR heteromers are differentially regulated by LOX metabolites. Further, in striking contrast to DOR and KOR, DOR-KOR heteromers appear to remain functionally competent for a prolonged period of time under inflammatory conditions, suggesting that they may be suitable targets for development of peripherally-restricted pain medications.


Poster 514. Opioids and other Analgesics

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 514.08/P21

Topic: D.08. Pain

Support: R01 DA021332
Title: Epigenetic regulation of spinal cord gene expression contributes to enhanced postoperative pain and analgesic tolerance after continuous opioid exposure

Authors: *P. SAHBAIE1,2, D.-Y. LIANG1,2, X.-Y. SHI1,2, Y. SUN1,2, J. CLARK1,2;
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Abstract: Opioid induced hyperalgesia (OIH), as well as, analgesic tolerance are unfavorable consequences of extended opioid use. Both typically resolve within days after cessation of morphine treatment. Post-surgical pain is prolonged if mice are previously exposed to opioids. We have shown earlier that among several implicated gene targets, expression levels of Bdnf (Brain-derived neurotrophic factor) and Pdyn (Prodynorphin) were closely related to OIH. The present study was carried out to investigate the epigenetic regulatory changes of Bdnf and Pdyn in supporting the enhanced incisional pain hypersensitivity after opioid exposure. The study went on to characterize morphine analgesic tolerance at several timepoints following surgery in groups exposed to morphine earlier. Mice were treated with ascending doses of morphine for 4 days and subsequently received hind paw incisions. Mechanical withdrawal thresholds were significantly decreased in the morphine plus incision group compared to morphine or incision alone groups. Analgesic tolerance to morphine was increased on days 3 and 6 after surgery in the morphine plus incision group compared to respective controls. Expression of Bdnf and Pdyn were increased on day 1 but only Pdyn levels were elevated on day 3 after surgery. ChIP (Chromatin immuoprecipitation) assays demonstrated that promoter regions of Pdyn and Bdnf were more strongly associated with aceH3K9 (Acetylated histone H3 Lysine9) after morphine plus incision treatment than morphine or incision alone groups. The selective TrkB (tropomyosin-receptor-kinase) antagonist ANA-12 reduced hyperalgesia when given spinally one or three days after surgery. Intrathecal treatment with the selective kappa opioid receptor antagonist nor-BNI had similar effects on pain sensitivity. The administration of ANA-12 or nor-BNI attenuated morphine analgesic tolerance on day 1, but only nor-BNI was effective on day 3 after surgery in opioid exposed group. The co-administration of histone acetyltransferase inhibitor anacardic acid daily with morphine resulted in reversal of OIH and further treatment attenuated enhanced hyperalgesia in the morphine plus incision group compared to controls. The present study shows histone modification of spinal genes contributes to enhanced postoperative pain and analgesic tolerance after continuous opioid exposure. Treatments blocking the differential expression of BDNF and dynorphin by inhibiting histone acetylation would have a potential in reducing postoperative pain, OIH and tolerance.

Disclosures: P. Sahbaie: None. D. Liang: None. X. Shi: None. Y. Sun: None. J. Clark: None.
Title: An allosteric modulator of the mu-opioid receptor promotes opioid-mediated antinociception

Authors: *T. M. HILLHOUSE¹, J. E. HALLAHAN¹, K. E. LIVINGSTON¹, C. MEURICE², M.-H. LI³, S. L. INGRAM⁴, J. R. TRAYNOR¹;
¹Pharmacol., Univ. of Michigan, Ann Arbor, MI; ²Pharmacol., Univ. of Pennsylvania, Philadelphia, PA; ³Neurolog. Surgery, Oregon Hlth. & Sci. Univ., Portland, OR

Abstract: Positive allosteric modulators (PAMs) of the mu-opioid receptor, such as BMS-986122, are compounds that bind to a site on the receptor that is distinct from the orthosteric site for endogenous opioid peptides and traditional opioid analgesics and enhance the activity of orthosteric agonists. Such mu-opioid receptor PAMs (mu-PAMs) could act as stand-alone analgesics and/or enhance the action of traditional opioid agonists and thereby reduce the level of opioid drug required to afford pain relief. In cells expressing the mu-receptor BMS-986122 is silent, i.e. it does not activate signaling downstream of the mu-opioid receptor. However, in membranes from both cloned cells expressing mu-opioid receptors and mouse brain membranes BMS-986122 enhances the binding affinity and potency and/or efficacy of a wide range of mu-opioid receptor orthosteric agonists, including endogenous opioid peptides, in a probe-dependent manner. The most robust increase in affinity and potency is seen with R(-)-methadone. Here we provide proof of principle that mu-PAMs can be effective in vivo by determining antinociceptive activity in the mouse using the hot-plate assay. In addition, opioid modulation of GABA synaptic transmission was monitored in periaqueductal gray (PAG) neurons. BMS-986122 given intracerebroventricularly (i.c.v.) caused a substantial increase in the antinociceptive activity of R(-)-methadone given i.c.v. or systemically and a moderate increase in the antinociceptive activity of systemic morphine. At higher doses BMS-986122 alone (i.c.v.) afforded a short-lived antinociception that was completely prevented by pretreatment of the mice with naloxone or the selective mu-antagonist beta-FNA. Furthermore, BMS-986122 promoted swim-stress induced antinociception that was blocked by pretreatment with naloxone. In slices of the PAG, BMS-986122 enhanced the ability of Met-enkephalin to inhibit presynaptic GABA release. These studies demonstrate that mu-PAMs enhance the activity of the mu-opioid receptor in vivo and so
increase the antinociceptive activity of opioid drugs and endogenous opioid peptides acting at the mu-opioid receptor.

**Disclosures:** T.M. Hillhouse: None. J.E. Hallahan: None. K.E. Livingston: None. C. Meurice: None. M. Li: None. S.L. Ingram: None. J.R. Traynor: None.

**Poster**

514. Opioids and other Analgesics

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**Program/#/Poster#:** 514.10/P23

**Topic:** D.08. Pain

**Support:** Ministry of Education, Science, and Culture Japan (No. 24791979)

Takeda Science Foundation

**Title:** BK channel in microglia as a promising molecular target for the treatment of opioid-induced hyperalgesia

**Authors:** *Y. HAYASHI, H. NAKANISHI;* Fac of Dent. Sci. Kyushu Univ., Fukuoka, Japan

**Abstract:** BK channels are the intracellular Ca2+ and voltage gated potassium channel. They are widely distributed throughout the nervous system to control neuronal excitability and neurotransmitter release. They are also expressed in electrically non-excitable cells such as cancer cells and immune cells, whereas little is known about their functions. Recently, we have found that large outward currents mediated by BK channels in the spinal microglia contribute to the initiation of neuropathic pain. In the present study, we have examined a possible involvement of microglial BK channels in opioid-induced hyperalgesia, because some evidence suggests the involvement of microglia in this event. Repeated morphine administration gradually enhanced pain sensitivity. At the same time, repeated morphine administration activated BK channels in microglia, but not in neuron, by generation of arachidonic acid and its metabolites through μ receptors. Morphine-induced hyperalgesia was significantly suppressed by BK channel inhibitor. The development of hyperalgesia was accelerated by intrathecal administration of morphine-primed wild-type, but not BK channel-deficient, microglia. Furthermore, the activation of BK channels promoted P2X4 receptor trafficking to the cell surface of microglia. These results indicate that BK channels in the spinal microglia also play an important role in the development
of opioid-induced hyperalgesia. Therefore, the BK channel is a potential molecular target for the treatment of both neuropathic pain and opioid-induced hyperalgesia.

Disclosures: Y. Hayashi: None. H. Nakanishi: None.

Poster

514. Opioids and other Analgesics

Location: Hall A

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Topic: D.08. Pain

Support: ARC Fellowship 110100297

Title: Examination of classical and non-classical opioid receptor binding of neuroimmune targeted agents using radioligands

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Abstract: Recent advances in opioid research has shown that opioid agonists cause significant reactivity in immunocompetent cells of the brain and spinal cord which is hypothesised to be key in mediating some unwanted side effects such as analgesic tolerance, hyperalgesia and reward processes. Pharmacologically inhibiting the immune reactivity, genetically removing TLR4, or (+)opioid antagonists significantly increases opioid analgesia and diminishes these unwanted pharmacodynamic responses. Here we present an independent replication of these opioid tolerance and hyperalgesia results in TLR null mutant animals. Additionally, this study set out to examine a range of key drugs that target this immune reactivity and to determine if they have any direct action at opioid receptors. Secondly, we assessed if TLR4 could modify [3H](-)naloxone binding kinetics. And thirdly, we assessed if a (+)opioid isomer binding site could be detected. Saturation binding studies were performed with increasing concentrations of [3H](-)naloxone, [3H]diprenorphine, and [3H](+)naloxone. Binding reactions were performed using HEK-μOR membrane, mouse brain membranes, mouse brain homogenate, or HEK-μOR, HEK-κ-OR, HEK-δ-OR, HEK-TLR4/MD-2 or RAW264.7 homogenates. Animal tissues were also sourced from wild type and TLR4 null mutant Balb/c mice. Our data demonstrated that of a range of examined neuroimmune active agents that only amitriptyline and WZ811 (CXCR4 antagonist) have direct
binding at the mu receptor but with no agonist activity. Brains from null mutant TLR4 animals displayed a small but significant increase in [3H]diprenorphine μOR binding affinity (lower Kd) and in the total number of binding sites (Bmax). No specific binding site for [3H](+)-naloxone and minimal specificity was observed for [3H](-)-naloxone in the nanoM to low microM concentration range in any of the models tested. Autoradiographic imaging will also be presented. Ongoing exploration of the characterisation of the specific binding site of (+)-naloxone will be discussed, along with unpacking of the disconnect between in vivo behavioural data and in vitro results.


Poster

514. Opioids and other Analgesics

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 514.12/P25

Topic: D.08. Pain

Support: ARC Fellowship 110100297

ARC Centre of Excellence CE140100003

Title: Nitric Oxide implicated in the reduced inhibitory response of the distal colon in mice lacking TLR2/4 receptors

Authors: *V. STAIKOPOULOS*1, E. A. H. BECKETT2, X. Z. ZHANG3, S. HENG3, M. R. HUTCHINSON2; 1Physiol., Univ. of Adelaide, Adelaide University, Australia; 2Physiol., 3Chem., Univ. of Adelaide, Adelaide, Australia

Abstract: Evidence suggests that the undesirable central side effects of opioid medications involve the activation of Toll-like receptors (TLRs) rather than the classical (mu, kappa, delta) opioid receptors. In addition to their expression within the CNS, TLRs are expressed within various cell types of the gastrointestinal (GI) tract, yet their functional role in this location remains unclear. The involvement of TLRs in opioid-induced inhibition of GI motility was investigated in vivo and in vitro using wildtype Balb/c and genetically TLR2/4 deficient male mice (TLR2/4-/-). In vivo, morphine-induced retardation of GI transit was significantly diminished in TLR2/4-/- mice by 57%. In organ bath experiments, a 10-fold higher concentration
of morphine was required to abolish migrating motor contractions (MMCs) at the distal end of TLR2/4/- colon (1uM) compared to the corresponding region of wildtype animals (0.1uM). Additionally, morphine potentiated the amplitude of EFS-evoked relaxations of Balb/c distal colon segments (6.2 ± 1.0 mN pre-morphine to 10.0 ± 1.0 mN in morphine, 10Hz EFS) but a similar potentiation of relaxation response was not seen in TLR2/4/- (4.18 ± 0.9 mN elicited by 10Hz EFS pre morphine and 2.67 ± 0.8 mN post-morphine). It has been suggested that attenuated TLR signalling in utero compromises the development of nitrergic nerves innervating the distal bowel. Our immunohistochemical analysis of distal colon segments of adult wildtype and TLR2/4/- failed to reveal a significant difference in either the number of Hu-positive nerve cell bodies per ganglia nor the proportion of nNOS-positive cell bodies to the total number of Hu-positive cell bodies. However, decreased intensity of nNOS immunofluorescent labelling within intramuscular terminals was observed. Relaxations elicited by 10uM concentrations of the exogenous nitric oxide donor, sodium nitroprusside (SNP) were attenuated in TLR2/4/- distal colon segments (relaxation to SNP (10 uM) 3.5mN in Balb/c and 1.7mN in TLR2/4/-) suggesting a reduction in the post-junctional sensitivity to nitric oxide accompanies loss of TLR2/4 signalling. Through the joint efforts of the ARC Centre of Excellence for Nanoscale Biophonotics and Institute for Photonics and Advanced Sensing, we are currently working towards the development of a photo-switchable sensor that will allow us to measure nitric oxide release in real time within gastrointestinal specimens. We are hopeful that the development of such a tool will assist with quantifying and localizing release of NO for this and other biological research applications.


Poster

514. Opioids and other Analgesics

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Topic: D.08. Pain

Support: CERC08

Title: Expression of an alternative delta-opioid receptor transcript in the mouse spinal cord

Authors: *M. H. PILTONEN, A.-J. CHABOT-DORÉ, M. PARISIEN, L. S. STONE, L. DIATCHENKO;
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Abstract: The delta-opioid receptor (DOR) is an opioid receptor whose endogenous ligands are enkephalins. Structurally, it has 7-transmembrane (7TM) domains, making it part of the G protein–coupled receptor family. Studies have linked DOR to pain perception, regulation of mood and neuroprotection. Although only one gene transcript is known to code for DOR in human and mouse, pharmacological characterization has led to classification of two receptor types: delta1 and delta2. The apparent simplicity of the DOR transcripts is surprising in the face of another closely related receptor, the mu-opioid receptor (MOR). MOR displays a diversity of transcripts; some encode for N-terminally truncated 6-transmembrane (6TM) domain receptors, while others are translated into single-TM domain chaperones, all in addition to the major 7TM isoform. Also, the human kappa-opioid receptor has a reported alternative transcript that is predicted to code for a 6TM receptor. Recently, we discovered an alternative transcript of the human DOR encoding for a 6TM receptor, which directed us to look at the mouse gene as well. While searching databases, we found an alternative DOR transcript for mouse in the NCBI library, annotated as a predicted transcript variant X1. Unfortunately, we found no literature regarding this transcript. Structurally, X1 consists of a fragment of exon 1, an additional exon A between exons 1-2, followed by exon 3. Exon A introduces a premature stop codon in respect to the canonical start site, but also incorporates a downstream second translational start site. Transmembrane domain prediction tool showed that it would yield a truncated 6TM receptor with an N-terminal peptide tail. From the spinal cord of a wild-type mouse, we were able to PCR amplify and sequence a long fragment of the X1, spanning from exon A to the last coding exon 3. Interestingly, the same fragment was found also in the DOR knockout mouse with a deletion of exon 1 (B6.129P2–Oprd1tm1Dgen). Our finding was supported by another experiment, in which we combined rapid amplification of 5’ ends PCR with deep sequencing of long transcripts derived from the mouse spinal cord. The same X1 transcript appeared in the sequence alignment. Taken together, our results provide a possible explanation for the existence of delta1 and delta2 receptor subtypes. It will be essential to study if the signaling of the 6TM DOR differs from that of the classic 7TM DOR, and to identify specific roles of these receptor types in normal physiology and disease pathophysiology. This will help to evaluate the potential of the receptors as a therapeutic drug targets, as there are no DOR-selective drugs available for clinical use yet.

Disclosures: M.H. Piltonen: None. A. Chabot-Doré: None. M. Parisien: None. L.S. Stone: None. L. Diatchenko: None.
Title: Awakening the delta opioid receptor in peripheral sensory neurons

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Abstract: Peripherally acting opioids are desirable for producing analgesia while eliminating debilitating central side effects. However, peripheral opioid efficacy is reduced in the absence of tissue inflammation, such that the delta opioid receptor (δ-, DOR) is less responsive to agonist activation. Bradykinin (BK), a potent inflammatory mediator, has been shown to enhance receptor responsiveness and increase peripheral opioid efficacy in a protein kinase C (PKC) dependent manner. Therefore, constitutively desensitized DOR becomes “primed” for activation following inflammation. A gap in knowledge exists concerning the mechanism regulating constitutive DOR analgesic incompetence and represents an important opportunity to identify new pharmaceutical targets to enhance opioid efficacy. Here we report that G protein-coupled receptor kinase 2 (GRK2) naively associates with DOR in sensory neurons. Conversely, BK pretreatment reduces this interaction. Moreover, siRNA-mediated knockdown of GRK2 in dorsal root ganglion (DRG) nociceptors resulted in a significant increase in functional DOR competence, independent of BK. Overexpression of GRK2 or a kinase inactive mutant blocked BK-induced DOR competence, and suggests that kinase activity is not necessary for GRK2 modulation of DOR. Given its role in DOR signaling, a change in GRK2 targeting in peripheral sensory neurons may enhance DOR competence. Raf-1 kinase inhibitory protein (RKIP) is an important signaling modifier. Following BK treatment, PKC promotes phosphorylation of RKIP and RKIP sequestration of GRK2 in primary sensory neurons. To determine the role of GRK2 sequestration by RKIP in functional DOR competence, DRG were nucleofected with RKIP or a phospho-deficient mutant. Overexpression of RKIP enhanced DOR activity compared to the mutant, and was further enhanced by pretreatment with BK. Conversely, RKIP siRNA treatment suppressed DOR activity. Thus, one mechanism responsible for functional DOR priming by BK is RKIP sequestration of GRK2. To further elucidate the role of GRK2 in DOR competence in vivo, we decreased GRK2 expression using targeted intrathecal antisense oligodeoxynucleotide (AS-ODN). GRK2 AS-ODN administration enhanced DPDPE inhibition of intraplantar PGE2-induced thermal allodynia relative to mismatch controls. Interestingly, the onset of DPDPE-induced analgesia occurred more quickly during GRK2 knockdown when pretreated with BK. Collectively, the results from these studies demonstrate that reducing chronic GRK2 association with DOR can significantly enhance DOR competence and improve analgesic efficacy. Research supported by NINDS NS082746 and NIDCR DE014318.
Implication of COPB1 in intracellular retention of the delta opioid receptor

Authors: *L. GENDRON, E. ST-LOUIS, J.-L. PARENT;
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Abstract: As opposed to most G protein-coupled receptors, the delta opioid receptor (DOP receptor) is poorly addressed at the plasma membrane. Indeed, it has been proposed that, under normal conditions, only a small proportion of DOP receptors can escape the endoplasmic reticulum (ER) and reach the plasma membrane. We and others have shown previously that the plasma membrane density of DOP receptors in neurons as well as the analgesic effects of delta agonists can however be increased by morphine and inflammation. Most recently, we have described an important role for cdk5 in mediating these effects. In this study, we sought to better described the molecular mechanisms involved in the regulation of DOP receptor trafficking. We have identified and investigated the role of 4 putative COPB1 binding motifs located in the intracellular loops (ICL) of the DOP receptor. In particular, one COPB1 binding motif was found within the consensus cdk5 phosphorylation motif in the ICL2 of the DOP receptor. In transfected HEK cells, the colocalization of DOP receptors with COPB1 was confirmed by confocal microscopy. When co-expressed in the same cells, we also found that COPB1-myc co-immunoprecipitated with Flag-DOP receptor. Using GST pull-down assays, we confirmed that COPB1 is able to interact with DOP receptor’s ICL2 and ICL3 via the COPB1 binding motifs and that the mutation of these motifs interferes with the ability of COPB1 to associate with DOP receptors. Most interestingly, replacing T161 (the ICL2 amino acid residue phosphorylated by cdk5) by a negatively charged residue (i.e. phosphomimetic) decreased the ability of COPB1 to
bind the ICL2 of the DOP receptor. Because COPB1 is known to be involved in the retrograde transport from the Golgi to the ER, we also studied the impact of mutations altering the COPB1 binding motifs on the surface expression of Flag-DOP receptors in HEK cells using an ELISA assay and found that one of the mutants had higher cell surface expression. Together, our results suggest that COPB1 is involved in the intracellular retention of DOP receptors. We now propose that cdk5 regulates the trafficking of DOP receptor by promoting the phosphorylation of T161, which in turn interferes with the association of COPB1, helping the receptor to escape the ER and reach the plasma membrane.


Poster

514. Opioids and other Analgesics

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Shirley and Stefan Hatos Research Foundation

Dept. of Psychiatry at UIC

Title: The delta opioid receptor agonist SNC80 preferentially recruits beta-arrestin 1 to promote analgesic tolerance

Authors: *A. VICENTE-SANCHEZ*, A. F. TIPTON, H. AKBARI, L. SEGURA, M. L. SMITH, A. A. PRADHAN;

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Abstract: Ligand directed signaling via the delta opioid receptor (DOR) has important implications given the potential therapeutic uses of delta agonists in the treatment of chronic pain and emotional disorders. We had previously shown that repeated injection of the high-internalizing delta agonist (+)-4-[(αR)-α-((2S,5R)-4-allyl-2,5-dimethyl-1-piperazinyl)-3-methoxybenzyl]-N,N-diethylbenzamide (SNC80), produced acute behavioral desensitization while the low-internalizing delta agonist N,N-diethyl-4-(phenyl-piperidin-4-ylidenemethyl)-benzamide (ARM390) did not. Since beta-arrestins are well known to regulate G protein-coupled receptors signaling and trafficking, we therefore investigated the behavioral significance of
ligand-specific interactions between beta-arrestin 1 and the DOR. Mice lacking beta-arrestin 1 showed enhanced and longer lasting pain-relieving effects of SNC80, and decreased acute tolerance following repeat exposure to the agonist. In contrast, ARM390 produced similar analgesic effects and no acute tolerance in both wildtype and knockout animals. Following chronic treatment, the absence of beta-arrestin 1 attenuated the extent of tolerance to SNC80, but not to ARM390. Furthermore, chronic treatment with SNC80 abolished delta agonist-induced GTPγS binding in wildtype brain membranes, whereas DOR-G protein coupling remained intact in beta-arrestin 1 knockout mice. Overall, these results indicate that delta opioid receptor agonists interact with beta-arrestins in a ligand-biased manner, and that the high-internalizing agonist SNC80 preferentially recruits beta-arrestin 1.

effects of CB2 receptor activation. Using protein-protein docking, we found a novel interaction between CB2 receptor and Gαs and confirmed it with co-immunoprecipitation. However, GNAS shRNA targeting Gαs didn’t inhibit CB2 receptor activation-induced β-endorphin production and AM1241 significantly attenuated the forskolin-stimulated cAMP accumulation. The Gi/o inhibitor pertussis toxin or the Gβγ inhibitor Gallein blocked CB2 receptor-induced β-endorphin production. Also, the ERK/MAPK kinase inhibitor PD98059, but not the PLC inhibitor U73122, abolished CB2 receptor activation-induced increases in β-endorphin production in cultured human keratinocytes cells. The elevation in intracellular Ca²⁺ occurred in parallel with CB2R activation-induced β-endorphin release. By using BAPTA-AM and thapsigargin to inhibit intracellular Ca²⁺, we demonstrated that CB2 receptor activation-induced β-endorphin release was calcium dependent. Using a rat model of inflammatory pain, we showed that the MAPK kinase inhibitor PD98059 abolished the peripheral effect of the CB2 receptor agonist on nociception. We thus present a novel mechanism of CB2 receptor activation-induced β-endorphin release through Gi/o-Gβγ-MAPK-Ca²⁺ signaling pathway. Our data also suggest that stimulation of MAPK contributes to the peripheral analgesic effect of CB2 receptor agonists.


Poster

514. Opioids and other Analgesics

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Support: Chaire de recherche du Canada en neurophysiopharmacologie de la douleur chronique

Modulation of G protein-coupled receptor trafficking and activity by receptor-interacting proteins from the Natural Sciences and Engineering Research Council of Canada (NSERC)

Title: Knockdown of the secretory protein secretogranin III results in a loss of NTS2 receptor-mediated spinal analgesia

Authors: *M. ROUX¹, M. LEMIRE², J. LAINÉ¹, J.-M. LONGPRÉ¹, A. M. JACOBI², S. D. ROSE², M. A. BEHLKE², P. SARRET¹;
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Abstract: Export of neo-synthesized G protein-coupled receptors (GPCRs) from intracellular compartments to the cell surface represents a crucial checkpoint in controlling the level of functional receptors available at the plasma membrane and the magnitude of the cellular response elicited by a ligand. Although cell-surface export of GPCRs is commonly considered to implicate the constitutive unregulated secretory pathway, there is now growing evidence indicating that their post-Golgi trafficking to the cell surface is dynamically regulated by molecular accessory escort proteins and may also rely on the non-canonical regulated secretory pathway via their packaging into large dense-core vesicles. However, the molecular mechanisms underlying GPCR transport to the plasma membrane remain poorly defined. Among GPCRs, we previously demonstrated that activation of the neuropeptide receptor 2 (NTS2) produces strong analgesia in different animal pain models. Despite its therapeutic potential, previous studies have shown that NTS2 is poorly expressed on the plasma membrane under basal conditions, but rather stored within the Golgi intracellular sorting compartment. In order to characterize the underlying mechanisms promoting the cell surface recruitment of NTS2, we first screened for interaction partners using a yeast two-hybrid system. This assay identified secretogranin III (SgIII), involved in the formation of regulated secretory vesicles as a NTS2-binding partner. The molecular association between SgIII and NTS2 was further supported by co-immunoprecipitation and GST pull-down assays, as well as by subcellular co-localization studies. As part of this project, we wished to further dissect the role of SgIII in NTS2 receptor function. To this aim, 27-mer Dicer-substrate siRNAs (DsRNA) used to knockdown SgIII were evaluated in the acute tail-flick nociceptive test, in which intrathecal injection of the NTS2-selective agonist, JMV-431 induces a dose-dependent antinociceptive response. Rats, pre-treated 48h and 24h prior behavioral experiments with DsRNA targeting SgIII, exhibited a 70% decrease of the JMV-431-induced analgesia. This result seems specific to NTS2 since the injection of SgIII DsRNA did not affect the antinociceptive effects of morphine or deltorphin II, acting respectively on µ opioid (constitutive secretory pathway) and δ opioid (regulated secretory pathway) receptors. In conclusion, these results demonstrate that the sorting of NTS2 through its interaction with SgIII along the regulated secretory pathway drives NTS2-mediated spinal analgesia.

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Effect of eugenol in histamine-induced itch and hapten-induced atopic dermatitis

Eugenol is extensively used in dentistry because of local analgesic reagent via inhibition of voltage-gated sodium channel (VGSC) and became known as has properties such as antioxidant and anti-inflammation. Also, eugenol has analgesic effect that mechanical allodynia by peripheral nerve injury were relieved by inhibiting of HCN channels. In the present study, we have examined effect of eugenol in histamine-induced itch and hapten-induced atopic dermatitis. Histamine-induced itch were reduced by eugenol cream as dose dependent manner. Also, eugenol and eugenol isoform (methyl eugenol, isoeugenol) has inhibited histamine-induced itch.

Next, we have examined function of eugenol using patch clamp. Eugenol has inhibited capsaicin-induced current at TRPV1-transfected HEK 293 cell. Next, we investigated that eugenol cream assists skin recovery in atopic dermatitis. When we applied eugenol cream for 1 week, improved tissue status and reduced cytokines (IL-4, IL-13) in tissue. Finally, we have tested effect of eugenol as behavioral test in atopic dermatitis model. Eugenol cream has inhibited itch response in hapten-induced atopic dermatitis. Taken together, these results revealed that eugenol is novel therapeutic agent in hapten-induced atopic dermatitis and histamine-induced itch.

Disclosures: S. Lee: None. P. Cho: None. J. Lim: None. S. Oh: None. S. Jung: None.
Title: Targeting putative mu opioid/chemokine receptor type 5 heteromers potently attenuates nociception in a murine model of chemotherapy-induced peripheral neuropathy

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Abstract: Although powerful pharmacological antineoplastic therapies has led to increased survival for millions of cancer patients, chemotherapeutic agents such as cisplatin produce a number of serious side effects, including peripheral neuropathy. Chemotherapy-induced peripheral neuropathy (CIPN) causes sensory disturbances in the extremities including numbness, paresthesia, and pain, and is the primary dose-limiting side effect that reduces efficacy of treatment and ultimately affects survival. Analgesics typically used to treat neuropathic pain appear to be relatively ineffective in alleviating pain from CIPN as well as display their own display dose-limiting adverse effects, such as those associated with opiates. Given the requisite need for superior pharmacological agents in its treatment, we investigated the antihyperalgesic effects of a novel bivalent ligand, MCC22, in a murine model of cisplatin chemotherapy-induced neuropathic pain. MCC22 contains both mu agonist and CCR5 antagonist pharmacophores linked through a 22 atom spacer. Given the existence of opioid receptor heteromers in cultured cells and possibly in vivo, MCC22 may be a potent alternative for the treatment of neuropathic pain without attendant side effects.

Adult male C3H/HeJ mice were tested for mechanical paw withdrawal responses on 2 consecutive days prior to treatment by determining the frequency of withdrawal evoked by a calibrated von Frey monofilament with a bending force of 3.9 mN applied to the plantar surface of the hind paws. Mice were then given 1 mg/kg of cisplatin intraperitoneal (i.p.) daily for 7 consecutive days and on day 14 post-injection, mechanical hyperalgesia was assessed. Compounds were administered intrathecally (i.t.) and i.p. weekly to determine peak time effects and ED50/80. Compared to the anti-hyperalgesic effectiveness of the standard opioid agonist morphine, we evaluated the effects of MCC22 both i.t. and i.p. and found MCC22 given i.t. (ED50: 0.0004 pmol/mouse (0.0002-0.0009 95% CI)) to be significantly more potent than i.t. morphine (ED50: 27.44 pmol/mouse (16.57-45.43 95% CI)) with a peak time of 20 minutes. MCC22 exhibited no tolerance and increased in potency over 115 days. It was also found to be significantly more potent when given i.p. (ED50: 3.07 pmol/mouse (2.45-3.84 95% CI)) compared to morphine (ED50: 14.28 pmol/mouse (12.70-16.07...
95% CI) with a peak time of 30 minutes and again did not exhibit tolerance. Morphine both i.t. and i.p. showed significant tolerance. MCC22 potently attenuates hyperalgesia in a cisplatin model of neuropathy and may offer a viable treatment for patients who suffer from CIPN pain without concommitment side effects.


Poster

514. Opioids and other Analgesics

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Topic: D.08. Pain

Support: CIHR/IRSC Grant

NSERC/CRSNG Grant

Title: β-arrestin-2-biased apelin receptor agonists as novel potent analgesics

Authors: *É. BESSERER-OFFROY, M. LAFRANCE, M. OUIRZANE, A. MURZA, J.-M. LONGPRÉ, É. MARSault, R. LEDUC, P. SARRET;
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Abstract: Apelin is the endogenous ligand of the class A G-protein coupled receptor APJ. We and others have recently demonstrated that spinal or supraspinal delivery of apelin-13 exerts potent analgesic effects in both acute and tonic pain paradigms. Accordingly, APJ is highly expressed in brain regions involved in the regulation of pain transmission and modulation, notably the dorsal root ganglia as well as the periaqueductal grey and rostroventral medulla. The present study was aimed to decode by which signaling pathways the apelin peptide exerts its antinociceptive activity. For this purpose, we synthesized a series of apelin-13 analogs exhibiting unnatural amino acids in position 12 and 13. Pro\superscript{12} and Phe\superscript{13} were respectively substituted by aminoisobutyric acid (Aib) and by the aromatic residues 1-Naphthylalanine (Nal) or 2-Nal. The antinociceptive activities of these newly synthesized compounds were evaluated in the experimental model of formalin-induced tonic pain in Sprague-Dawley rats. We also determined, their ability to trigger different signaling pathways such as engagement of G-proteins G\subscript{i1}, recruitment of β-arrestins 1 and 2, and inhibition of cAMP production, following binding to APJ. We then quantified bias using the Black and Leff operational model by calculating the
transduction ratio for each pathway in order to identify potential biased APJ agonists. Our results revealed that all the tested compounds were less active than apelin-13 to inhibit forskolin-induced cAMP production with EC\textsubscript{50} 10- to 20-fold higher than the native peptide. However, only three analogs were 2- to 8-fold more potent than apelin-13 to recruit β-arrestin 2. Interestingly, these compounds were also the ones to reverse the spontaneous pain behaviors elicited by intraplantar formalin. These analogs present a unique and conserved biased signaling profile towards β-arrestin 2 recruitment over inhibition of cAMP production with significant bias factors ranging from 35- to 83-fold. Taken together, our results demonstrate that incorporation of unnatural amino acids at the C-terminal end of the apelin peptide impacts on the APJ receptor signaling signature and that β-arrestin 2 biased APJ agonists elicit potent analgesia. These results also emphasize the involvement of the apelinergic system in central modulation of pain and represent valuable information for future development of analgesics targeting the APJ receptor.


Poster

514. Opioids and other Analgesics

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Topic: D.08. Pain

Support: Canadian Institutes of Health Research (CIHR)

Title: Site-selective modifications of the neurotensin hexapeptide fragment lead to the generation of highly active and metabolically stable NT(8-13) analogs

Authors: *M. VIVANCOS\textsuperscript{1}, E. BESSERER-OFFROY\textsuperscript{1}, R. BROUILLETTE\textsuperscript{1}, A. RENÉ\textsuperscript{2}, R. FANELLI\textsuperscript{2}, M. LAFRANCE\textsuperscript{1}, P. TÉTREAULT\textsuperscript{1}, J. COLERETTE-TREMBLAY\textsuperscript{1}, J.-M. LONGPRÉ\textsuperscript{1}, J. MARTINEZ\textsuperscript{2}, F. CAVELIER\textsuperscript{2}, P. SARRET\textsuperscript{1};\textsuperscript{1}Pharmacol. and Physiol., Univ. De Sherbrooke, Sherbrooke, QC, Canada; \textsuperscript{2}IBMM, UMR-CNRS-5247, Univ. de Montpellier, Montpellier, France

Abstract: The field of peptide-based therapeutics and diagnostics is experiencing renewed interest during the last decade, when compared to small molecule therapies. However, despite their excellent safety, tolerability and efficacy profiles, naturally occurring peptides are often not directly suitable for use as future therapeutics because they have intrinsic weaknesses, including poor physicochemical stability, and a short circulating plasma half-life. In the present study, we
developed several strategies to increase the chemical stability and reduce the enzymatic degradation of the endogenous neurotensin hexapeptide fragment (NT(8-13)). To this aim, we synthesized a series of NT(8-13) analogs harboring site-specifically modified unnatural amino acids and reduced amide bonds. Pro10 and Leu13 were respectively substituted by silaproline (Sip) and (trimethylsilyl)alanine (TMSAla) unnatural amino acid residues and a reduced amide bond was incorporated between the Lys8-Lys9 amino acid pair. Our results revealed that these new NT(8-13) analogs bind with high affinity to both NTS1 and NTS2 receptors and exhibited improved plasma stability, with half-life exceeding 1 hour. We also determined, in vitro, their ability to trigger different signaling pathways linked to NTS1 activation. To this end, we used a BRET-based biosensor assay to monitor β-arrestins and G-protein engagement. A few of these compounds were found to be more effective in promoting β-arrestin and G-protein activation than the native NT peptide. We also demonstrated that these newly synthetized analogs were highly potent in reversing carbachol-induced ileal smooth muscle contractions in isolated organ bath. In in vivo assays, some of these NT(8-13) derivatives were shown to exert pronounced and sustained hypotensive and/or hypothermic actions. We finally tested their ability to reduce the pain sensations in the acute thermal tail-flick test and in the formalin-induced tonic pain model. Our results revealed that all the tested compounds were more potent than NT(8-13) to produce analgesic responses in the tail-flick assay and to abolish the formalin-evoked spontaneous nociceptive behaviors. Altogether, these results demonstrated that the chemically modified NT(8-13) analogs exhibit improved therapeutic profiles and may represent a promising avenue to regulate the physiological functions of the neurotensinergic system.


Poster

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Support: NIH: 1R21AI113580-01

Title: Botulinum neurotoxin derivatives as pain specific inhibitors
Abstract: Background: Botulinum neurotoxins (BoNTs), which consist of a heavy chain (HC) responsible for uptake and a light chain (LC) responsible for catalytic activity, when given intrathecally (IT), produce long term alterations in pain initiated by inflammation and nerve injury in animal models. The HC uptake mechanisms confer no cellular specificity. BoNTs can enter and block neurotransmission in any neuron. The use of IT BoNTs for pain treatment is therefore beset with serious side effects such as muscle paralysis and the potential for a heightened pain state due to a block of inhibitory neurons. For this study, we developed a recombinant BoNT LC coupled to substance P (SP) that specifically targets sensory neurons involved in pain processing by enhancing clinical efficacy and safety. Therefore our aim was to test the efficacy of a recombinant SP-LC in *in vitro* using cell culture and in *in vivo*, using the intraplantar formalin model of facilitated processing. Methods: The truncated BoNT-A1 LC was chemically linked to SP-cys. Following exposure to cultured primary rat spinal cord (RSC) and dorsal root ganglion (DRG) cells as well as NK1 receptor expressing cell line (NG108-15), cleavage of SNAP-25 (SNARE protein) was analyzed. The effect of IT SP-LC was tested using mice with the formalin evoked flinching model. Results: Exposure of NG108-15 for 24 or 48 h resulted in uptake of SP-LC and cleavage of the SNARE protein SNAP-25 inside the cells in a concentration-dependent fashion. Mice injected IT (percutaneously) with SP-LC resulted in a significant reduction in Phase II but not Phase I flinching as compared to mice injected with saline, indicating an inhibition in central activation. Measurement of tactile allodynia by von Frey hairs 5, 6, and 7 days post formalin test revealed a prominent tactile allodynia in vehicle mice, but this was reduced in mice injected with SP-LC. Conclusion: These data suggests that SP-LC can enter NK1 receptor bearing cells and is catalytically active inside these cells as indicated by SNAP-25 cleavage in sensory neurons and thereby reduces central activation after induction of pain in mice. This indicates that BoNT can be tailored to selectively target the cells of interest and can be a promising approach towards the development of pain therapy. (NIH: 1R21AI113580-01)

The granulocytes-derived chemokine bv8/pk2 is involved in g-csf induced pain

Authors: *L. NEGRI, Prof. R. LATTANZI;
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Abstract: Granulocyte-colony stimulating factor (G-CSF) is commonly employed to reduce the risk of chemotherapy-induced neutropenia in cancer patients, however pain is relevant side effect. G-CSF is the major inducer of the chemokine Bv8/PK2 in inflammatory granulocytes. Bv8/PK2, through GPCR, PKR1 and PKR2, produces pro-algesic and pro-inflammatory effects. Receptors and signaling mediators of G-CSF are functionally expressed on DRG neurons.

Aim: To verify, in mice, whether thermal hyperalgesia induced by intraplantar (i.pl.) administration of G-CSF is mediated by Bv8/PK2 system. Methods: We evaluated thermal hyperalgesia (TH, plantar-test), induced by i.pl. increasing doses of G-CSF, in WT, PKR1-KO and PKR2-KO mice and the ability of a PKR1-antagonist (PC1) and of a TRPV1 antagonist (NF1-56 HCl) to abrogate G-CSF-induced thermal hyperalgesia. Number of circulating granulocytes (Burker-chambre), PK2-mRNA (RT-PCR) and granulocyte-recruitment (myeloperoxidase-assay) in G-CSF or saline injected paw were assessed 4 h after injection.

Results: In WT and in PKR2-KO mice G-CSF (300ng) induced TH for 8h, whereas PKR1-KO mice were significantly less sensitive (>1 mcg vs 300ng ). PC1 (50 ng) antagonized G-CSF-induced TH in WT and in PKR2-KO mice, whereas it was ineffectuous (up to 500ng) in PKR1-KO mice. The TRPV1 antagonist, NF1-56-HCl (50ng), antagonized TH in all three genotypes.


Disclosures: L. Negri: None. R. Lattanzi: None.
Abstract: Chemotherapy-induced peripheral neuropathic pain (CIPNP) is a severe dose-limiting side effect of widely used cytostatics, such as taxanes, platinum derivates and vinca-alkaloids. The mechanisms of CIPNP are poorly understood but seem to involve increased activity of ligand gated ion-channels, such as TRP channels in sensory neurons. Several endogenous TRP-channel modulators have already been identified among the group of oxidized lipid mediators. Here, we report involvement of oxidized lipids in Taxane-evoked CIPNP. Lipidomic analyses revealed increased synthesis of the oxidized linoleic acid metabolite 9,10-EpOME in sensory neurons of mice following paclitaxel treatment causing sensitization of TRPV1 via a cAMP-PKA-mediated mechanism, resulting in increased TRPV1 dependent sEPSC-frequency in lamina II neurons in the dorsal horn of the spinal cord. Moreover, treatment of sciatic nerves and DRG-neurons with 9,10-EpOME leads to increased release of the proinflammatory peptide CGRP, and peripheral injection of this lipid causes a reduction of both mechanical and thermal thresholds in wild type but not TRPV1-deficient mice. We conclude that synthesis of oxidized lipids in sensory neurons contributes to paclitaxel-induced CIPNP and inhibition of lipid oxidizing pathways may be a strategy for the development of novel analgesics.
Support: NINDS DP2NS087725-01

Whitehall foundation

Title: The role of multi-whisker integration during active sensation to the cortical representation of somatotopic space

Authors: *S. PLUTA¹, E. H. LYALL², E. S. RYAPOLOVA-WEBB³, G. I. TELIAN¹, H. ADESNIK¹;
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Abstract: The receptive field of cortical neurons are formed by a complex integration of feedforward and horizontal input. The somatotopic, columnar organization of the rodent barrel cortex provides a powerful model for testing the relative contribution of these inputs to the neural representation of space. When performing an object discrimination task, rodents palpate the object with multiple whiskers, and their performance is greatly reduced or abolished when the whisker pad is experimentally trimmed to only a single whisker. Nonetheless, the role of multi-whisker integration to the cortical representation of space is not well understood, particularly in an awake, actively sensing animal. We addressed this problem by developing a novel method in the awake-behaving mouse for determining the contribution of principal (primarily feedforward) and surround (primarily horizontal) whisker input to the cortical representation of space. We found that most neurons can be categorized by two major groups: (1) surround-whisker facilitated and (2) surround-whisker suppressed, while a smaller subset of neurons were both facilitated and suppressed as a function of object location. Recording activity simultaneously across multiple barrel columns reveals how a population code derived from the multi-barrel distribution of firing rates could discern object location.


Poster

515. Somatosensory Cortex

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 515.02/P40

Topic: D.09. Tactile/Somatosensory Systems

Support: NINDS Grant DP2NS087725-01
NEI grant R01EY023756-01
Title: A direct translaminar inhibitory circuit tunes cortical output

UC Berkeley, Berkeley, CA

Abstract: Anatomical and physiological experiments have outlined a 'canonical' blueprint for the feed-forward flow of activity in cortical circuits: signals are thought to propagate primarily from the middle cortical layer, L4, up to L2/3, and down to the major cortical output layer, L5. Pharmacological manipulations, however, have contested this model and suggested that activity in L4 may not be critical for sensory responses of neurons in either superficial or deep layers. To address these conflicting models we reversibly manipulated L4 activity in awake, behaving mice using cell type specific optogenetics. In contrast to both prevailing models, we show that activation of L4 directly suppresses L5 through deep, fast spiking inhibitory neurons. Our data suggest that the net impact of L4 activity is to sharpen the tuning of L5 neurons. Thus we establish a novel translaminar inhibitory circuit in the sensory cortex that acts to enhance the feature selectivity of cortical output.


Poster

515. Somatosensory Cortex

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

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Topic: D.09. Tactile/Somatosensory Systems

Support: The EPFL Blue Brain Project Fund

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CADMOS: The financial support for CADMOS and the Blue Gene/Q system is provided by the Canton of Geneva, Canton of Vaud, Hans Wilsdorf Foundation, Louis-Jeantet Foundation, University of Geneva, University of Lausanne and EPFL.

European Union Seventh Framework Program (FP7/2007-2013) under grant agreement no. 604102 (HBP)
Calculations were performed on the EPFL Blue Brain IV BlueGene/Q supercomputer hosted at the Swiss National Supercomputing Center (CSCS) in Lugano.

Title: Predicting the spatial resolution of thalamocortical input from a detailed model

Authors: *M. W. REIMANN, E. MULLER, H. MARKRAM;
Blue Brain Project, Geneve, Switzerland

Abstract: One distinctive feature of primary sensory areas in the neocortex is topographical mapping of inputs, e.g. locations of light stimuli on the retina, or the location of a touch. While it is easy to measure the resolution of the final mapping, it is unclear how this emerges during different stages of sensory processing. We investigated one stage of the process: i.e. the ability of neocortical microcircuits to discriminate thalamic input. We subjected a highly detailed model of a neocortical microcircuit of a rat to simulated thalamic input centered around spatial locations with different separations and tested the responses of neurons for significant differences in terms of their firing rate and the precise timing of their firing. The result was a map of the ability of individual cortical neurons to discriminate between spatially constrained thalamic input, dependent on stimulus separation and amplitude, cortical layer and the activity state of the network. We found that many neurons showed significant discriminatory power for separations over 50 µm, already when only the delay or the firing rate of their response was taken into account. Neurons with strong discriminatory power were found predominately in layers 5 and 6. Finally, we investigated how the measured spatial resolution relates to the ability to discriminate between more complicated spatio-temporal input patterns.

Disclosures: M. W. Reimann: None. E. Muller: None. H. Markram: None.

Poster

515. Somatosensory Cortex

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

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Topic: D.09. Tactile/Somatosensory Systems

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European Union Seventh Framework Program (FP7/2007-2013) under grant agreement no. 604102 (HBP)

Calculations were performed on the EPFL Blue Brain IV BlueGene/Q supercomputer hosted at the Swiss National Supercomputing Center (CSCS) in Lugano

Title: Predictive in silico reconstruction of cell-type specific synaptic anatomy and physiology in the neocortical microcircuit

Authors: *S. RAMASWAMY, J. G. KING, E. MULLER, J. RAHMON, M. REIMANN, H. MARKRAM; EPFL - Blue Brain Project, Geneva, Switzerland

Abstract: The neocortical microcircuit contains a rich diversity of excitatory and inhibitory neurons. Although previous research has revealed the fundamental characteristics of neocortical neuron types, knowledge of their axonal, dendritic, synaptic, and laminar organization remains very limited. The Blue Brain Project has developed a data-driven process for the in silico reconstruction of a neocortical microcircuit. The reconstruction integrates a vast array of biological data on neuronal morphologies and electrical types, ion channel kinetics and axo-dendritic density distributions, synaptic kinetics and dynamics from juvenile rat somatosensory cortex. These data were used to parameterize and validate the in silico anatomy and physiology of monosynaptic connections arising from the incidental axo-dendritic overlap of diverse pre- and post-synaptic morphological types. Experimental protocols of whole-cell paired recordings and glutamate uncaging under high extracellular Ca\textsuperscript{2+} concentrations in vitro were mimicked in silico to uncover a gamut of predictions on the anatomy and physiology of the synaptic connections of principal neocortical neuron types. The reconstruction predicts the complete intrinsic anatomical and physiological wiring diagrams of a microcircuit of neocortical neurons, in depth anatomy and physiology of nearly 2000 inter- and intra-laminar monosynaptic pathways, and functional synaptic maps for 55 identified neuron types based on afferent and efferent morphological, electrical, and synapse types. In addition, the reconstruction also predicts the physiology of synaptic transmission under low extracellular Ca\textsuperscript{2+} conditions observed in vivo. Component models of single neurons, synaptic transmission with stochastic transmitter release, and pairs of synaptically connected neurons of the in silico reconstruction are publicly available through the Neocortical Microcircuit Collaboration Portal (https://bbp.epfl.ch/nmc-portal) for community use and collaborative refinement. The spectrum of predictions obtained from the reconstruction can be challenged and validated through the design of targeted experiments. The resulting validated reconstruction can fill gaps in our knowledge on the precise roles of local synaptic transmission and neuronal function in modulating global circuit dynamics. The in silico reconstruction is therefore proposed as a complementary approach to current experimental techniques for the systematic characterization of neocortical synaptology.

Poster

515. Somatosensory Cortex

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 515.05/Q1

Topic: D.09. Tactile/Somatosensory Systems

Support:
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Calculations were performed on the EPFL Blue Brain IV BlueGene/Q supercomputer hosted at the Swiss National Supercomputing Center (CSCS) in Lugano

European Union Seventh Framework Program (FP7/2007-2013) under grant agreement no. 604102 (HBP)

Title: Data-driven construction of mouse whole-brain models

Authors: C. EROE, D. KELLER, H. MARKRAM, *M.-O. GEWALTIG; EPFL - Ctr. For Brain Simulation, Geneva, Switzerland

Abstract:
We present a semi-automatic process for constructing whole-brain models at the point neuron level from different sets of image stacks. Our process has two parts, each with several steps. In the first step, we determine the positions of all cells, using high-resolution Nissl stained microscope image stacks Allen Mouse Brain Reference Atlas [1]. In the second step, we determine the type of each cell, using in situ hybridization (ISH) image data from the Allen Mouse Brain Atlas. To increase the spatial resolution of the data, we re-aligned the ISH images to the reference atlas. Here we present differentiation into glia, excitatory neurons and inhibitory neurons. More fine-tinned differentiation is possible by adding more genes to this step. We compare the resulting cell and neuron numbers to literature data [2,3]. Our estimates of cell numbers are best for the isocortex (relative error 5%) and worst for the cerebellum (60%), where the cell density in the images is too high to distinguish individual cells. After differentiating glia...
and neurons, our estimates differ from the literature value by an average of 12% for the isocortex and 63% for the cerebellum. In the next step, we use two-photon tomography images of rAAV labeled axonal projections from the Allen Mouse Connectivity Atlas to determine the mesoscale connectivity between the neurons in different brain regions. For this step, a comprehensive comparison to experimental data is difficult, due to lack of comparable connectivity data. Finally, we obtain a network model that can be simulated with state-of-the art simulators like NEST and NEURON. We show results from a simulated whisker stimulation experiment and compare the evoked activity patterns to data from comparable calcium imaging experiments. We also present results from our ongoing research on reconstructing the microscale connectivity between the neurons in a small volume from appropriate data-sets. [1] Allen Mouse Brain Atlas (Reference Atlas Version 2 (2011)) , Allen Mouse Connectivity Atlas, http://www.brain-map.org [2] Herculano-Houzel S et al. Front. in Neuroanatomy 2013; (DOI:10.3389/fnana.2013.00035) [3] Herculano-Houzel S et al. Brain Behav Evol 2011;78:302-314 (DOI:10.1159/000330825)


Poster

515. Somatosensory Cortex

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

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Topic: D.09. Tactile/Somatosensory Systems

Support: The EPFL Blue Brain Project Fund

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European Union Seventh Framework Program (FP7/2007-2013) under grant agreement no. 604102 (HBP)

Calculations were performed on the EPFL Blue Brain IV BlueGene/Q supercomputer hosted at the Swiss National Supercomputing Center (CSCS) in Lugano.

Title: Data-driven in silico reconstruction of rat somatosensory cortex: Comparison to recent in vivo findings

Authors: *E. B. MULLER, G. CHINDEMI, T. NEWTON, M. NOLTE, S. RAMASWAMY, M. W. REIMANN, H. MARKRAM;
EPFL - Blue Brain Project, Geneva, Switzerland
Abstract: Despite recent experimental advances in cortical anatomy and physiology, the functional significance of cortical laminar architecture and the diversity of neuronal morphologies, electrophysiology, and synaptic interactions for cortical information processing remains largely a mystery. The Blue Brain Project has developed a data-driven in silico reconstruction of juvenile rat somatosensory cortex which integrates the current state of experimental knowledge on the anatomy and physiology of the microcircuit at the cellular and synaptic levels of organization. The in silico reconstruction contains 6 synapse types, and 55 morphological neuron types, 11 electrical types, 207 morpho-electrical sub-types, distributed according to experimentally observed proportions across the 6 cortical layers, optimized to fit somatic patch-clamp recordings to standardized protocols, and simulated in full morphological detail. Synaptic anatomy and physiology of the reconstructed microcircuit are presented in a companion abstract (Ramaswamy et. al.). While most integrated experimental data employed in vitro techniques on acute cortical slices, simulation of the reconstruction shows a spectrum of emergent network activity states. Baseline extracellular Ca2+ concentration is found to control the balance of excitation and inhibition in the network, and can be adjusted towards a state of criticality where bursting events have a range of amplitudes and frequencies around Ca2+ levels. We performed a range of in silico experiments to compare this critical network state to recent in vivo findings on spontaneous and evoked activity of cortical circuits. In silico results were found to be qualitatively comparable to their experimental counterparts without parameter tuning, and enabled predictions of the mechanisms at play beyond what was possible using current experimental techniques.


Poster
515. Somatosensory Cortex

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 515.07/Q3

Topic: D.09. Tactile/Somatosensory Systems

Support: The EPFL Blue Brain Project Fund

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CADMOS: The financial support for CADMOS and the Blue Gene/Q system is provided by the Canton of Geneva, Canton of Vaud, Hans Wilsdorf Foundation, Louis-Jeantet Foundation, University of Geneva, University of Lausanne and EPFL
Title: Recent improvements towards the accurate modeling of the rat brain using detailed morphologies on supercomputing technologies

Authors: *J. G. KING*\(^1\), F. DELALONDRE\(^1\), B. MAGALHAES\(^1\), P. KUMBHAR\(^1\), T. EWART\(^1\), A. OVCHARENKO\(^1\), S. YATES\(^1\), F. CREMONESI\(^1\), A. DEVRESSE\(^1\), M. HINES\(^2\), E. MULLER\(^1\), H. MARKRAM\(^1\), F. SCHUERMANN\(^1\);

\(^1\)Blue Brain Project, Brain Mind Institute, EPFL, 1202 Geneva, Switzerland; \(^2\)Dept. of Neurobio., Yale Univ., New Haven, CT

Abstract: The Blue Brain Project (BBP) aims at developing models and software to support the building and simulation of large-scale morphologically detailed and biologically accurate models of neocortical tissue that include hundreds of millions of neurons. Such an endeavor must leverage state-of-the-art high performance computing technology to attain the desired level of detail and model fidelity with a usable time-to-solution. Here we describe our recent developments towards the provision of a stack of scientific software to be made available to the Human Brain Project community. To improve our circuit building process, additional parameters have been added to better capture biological features of interest. Proximal contacts between neurons, or “touches”, are allowed some degree of variability in the space between cells. This space has been considered an allowance for spines along the morphologies and a recent additional filter now limits those spine lengths such that the final distribution matches those observed in tissue. Next, simulating the built circuit requires development of scalable software for supercomputing architectures to manage runtime. Our new simulation package, CoreNeuron, consists of the core functionalities of the NEURON software refactored using OpenMP threading, vectorization, and efficient memory layout. On leading-class supercomputing system the JUQUEEN IBM Blue Gene/Q, this implementation utilizes 458,752 computing cores and 1,835,008 threads to model a network made of 155 million neurons. Further developments have been carried out in conjunction with the Dynamic Exascale Entry Platform (DEEP). Such a platform consists of an x86-based “Cluster” with InfiniBand interconnect to run less scalable parts of an application while an Intel MIC based “Booster” with Terabit EXTOLL interconnect runs highly scalable compute kernels. Following code analysis, CoreNeuron computing needs were mapped to the appropriate parts of the heterogeneous DEEP system for increased node performance. Finally, to make these new features and optimizations accessible to users and ensure reproducibility, an automated continuous integration and deployment infrastructure is installed which makes all versions of software available to users.
Abstract: Somatosensory information from the limbs reaches the contralateral (contra) primary sensory cortex (S1) with a delay of 23ms for finger, and 40ms for leg (somatosensory N20). Upon arrival of this input in the cortex, motor evoked potentials (MEPs) elicited by transcranial magnetic stimulation (TMS) are momentarily inhibited. This phenomenon is called short-latency afferent inhibition (SAI) and can be used as a tool for investigating sensorimotor interactions in the brain. Here we tested whether SAI could also be elicited in the hemisphere ipsilateral (ipsi) to the sensory stimulated limb (ipsi SAI) and determined the precise onset of inhibition in the ipsi primary motor cortex (M1). We electrically stimulated the limb either contra or ipsi to the hemisphere receiving TMS, using a range of different interstimulus intervals (ISI). In Exp 1 (n=23), we tested finger muscles (first dorsal interosseous, FDI) and elicited robust contra SAI for ISIs corresponding to N20+7ms (p<0.001) and N20+22ms (p<0.001). By contrast, significant ipsi SAI was only observed for ISI N20+22ms (p<0.05). This pattern of outcome occurred regardless of whether the dominant or non-dominant hemisphere was tested. In Exp 2 (n=15), contra SAI was detected in the tibialis anterior muscle (TA) when stimulation occurred at ISIs corresponding to N20+5ms (p<0.001) and N20+10ms (p<0.001), but not at corresponding timepoints in the ipsi leg. In a third experiment (n=12) we directly compared ipsi SAI between hand and leg muscles for a large range of ISIs. No ipsi SAI was detected in TA, while ipsi SAI was present in FDI for ISIs between N20+15 to N20+40ms with strongest group-level reductions in MEP size at N20+20ms. Our data suggests a transcallosal route for ipsi sensorimotor integration for hand but not for leg muscles. The early emergence of ipsi SAI at
N20+20ms is consistent with the timing expected by a direct S1-S1 or contra S1-ipsi M1 pathway, precluding the involvement of much slower S2 interhemispheric communication. The absence of ipsi SAI in TA suggests that sensory input to the lower limbs may be processed predominantly contralaterally, as no evidence was found for ipsi sensorimotor integration.


Poster

515. Somatosensory Cortex

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 515.09/Q5

Topic: D.09. Tactile/Somatosensory Systems

Support: FIRCA Grant NS059061

NIH Grant NS044375

Hungarian Scientific Research Fund, OTKA NN79366

Title: Complementary role of intra- and inter-areal cortical connections in somatosensory processing in primates

Authors: *L. NEGYESSY1,2, E. PÁLFI1, M. ASHABER2, L. ZALÁNYI1, C. T. PALMER1, O. KÁNTOR2, R. M. FRIEDMAN4, A. W. ROE4;
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Abstract: The multiple body representation within somatosensory cortex provides a good opportunity to study structural and functional correlates of areal diversification and ultimately cerebral cortical functionality at a microscopic scale. Area 3b and area 1, two closely related regions, play critical roles in tactile perception and each possess an enlarged hand representation. We tested the hypothesis that the horizontal and laminar distribution of anatomical connectivity exhibit differential characteristics similar to some known functional differences between the two areas as for instance receptive field size and cortical magnification. We used bidirectional tract tracing via injections of BDA into distal finger pad representations of area 3b and area 1 of the squirrel monkey. The sizes of injections (about 300 µm in diameter) matched the size of
submodality specific tactile modules in areas 3b and 1. Irrespective of the origin, both retrograde and anterograde labeling exhibited supragranular dominance, making these cortical layers the main site of interactions between the two areas. Kernel density analysis of the lateral extension of retrograde labeling suggests that similarly sized neuronal populations provide input to column-size cortical regions within and between areas 3b and 1. However, considering the different magnification factors the cortical territory with high density labeling represents a larger skin area in area 1 than in 3b. Anterograde labeling revealed an anisotropic distribution of terminal axon arborizations that extended across the finger representations in both areas. Interestingly, terminal axon arborizations spread over a larger distance within an area when compared to that of the inter-areal projections. The broad interdigit interactions within an area may serve as the means for multidigit integration at single hierarchical levels, while homotopic distal finger pad representations mediate fast information exchange between areas via the existing thick axonal processes. The results point to the importance of intra-areal connections in integrative processing and the specialization of some inter-area connections for fast information exchange. Supported by: FIRCA NS059061 (to A.W.R. and L.N.), NS044375 (to A.W.R.) and the Hungarian Scientific Research Fund OTKA NN79366 (to L.N.).


Poster
515. Somatosensory Cortex

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 515.10/Q6

Topic: D.09. Tactile/Somatosensory Systems

Support: JSPS KAKENHI Grant Number 17500205

Title: Visual responsiveness of neurons in the secondary somatosensory area and its surrounding parietal operculum regions in awake macaque monkeys

Authors: *M. TAOKA1,2, S. HIHARA1,2, M. TANAKA1,2, A. IRIKI1,2;
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Abstract: It is believed that the secondary somatosensory cortex (SII), located in the upper bank of the lateral sulcus (LS), is principally engaged in the processing of somatosensory information. Previous neurophysiological studies performed on awake macaque monkeys demonstrated the
existence of neurons responsive to visual stimuli in the upper bank of LS caudal to the SII; however, sensory inputs other than somesthetic information have not been reported in the SII. In contrast, recent human brain imaging studies have revealed the effects of visual and auditory stimuli on SII activity, which suggest multisensory integration in the human SII. To determine whether multisensory responses of the SII also exist in nonhuman primates, we recorded single-unit activity in response to visual and auditory stimuli from the SII and surrounding regions in eight hemispheres from six awake macaque monkeys. We used three types of visual stimulation in this study: 1) moving the experimenter’s hand in front of the animal within its reach range (<40cm); 2) the same stimuli outside the reach range; and 3) observations of human actions (such as “reaching for a food container and picking up a piece of food”). Among the 1157 recorded neurons, we found 306 neurons that responded to visual stimuli. They rarely responded to simple object presentations (the static presentation of the experimenter’s hand, 1.6%), but did respond to rather complex stimuli, such as the stimulation of the peripersonal space (40.5%), observation of human action (29.1%), and moving-object stimulation outside the monkey’s reach (23.9%). These visual neurons often responded to somatosensory stimuli (37%) and monkeys’ self-initiated reaching and grasping actions (26%). We were able to test the responsiveness of a fraction of the visual neurons (about one-third of the 306 neurons) to auditory stimuli and found 10 neurons showing auditory responsiveness. Furthermore, all of them responded to somatosensory stimuli (trimodal neurons). Next, we investigated the distribution of the visual neurons in the LS upper bank. The visual neurons were usually recorded along with somatosensory responsive neurons that are distributed throughout the upper bank of LS; from the regions caudally bordering with area 7b/Ri (retroinsular area) to the region rostrally bordering with the PR (parietal rostroventral area) covering the entire SII. These results clearly show that the SII of macaques is a multisensory area. This emphasizes the need for a novel definition of the functional role of the SII beyond the currently attributed sensorimotor function arising from the idea that the SII is a unimodal somatosensory area.


Poster

515. Somatosensory Cortex

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 515.11/Q7

Topic: F.01. Human Cognition and Behavior

Support: ULB-Fonds Erasme (Brussels)
Title: Human secondary somatosensory cortex detects tactile novelty under the predictive coding theory

Authors: G. NAEIJE, V. WENS, B. MARTY, T. VAULET, M. OP DE BEECK, S. GOLDMAN, *X. DE TIÈGE;
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Abstract: This study investigates using magnetoencephalography the spatio-temporal dynamics and the neural mechanisms of somatosensory mismatch negativity (sMMN) under the predictive coding framework. Somatosensory evoked magnetic fields (SEF) were recorded in 16 right-handed adults while they underwent: (1) Oddball: 100 blocks of four standards followed by a deviant stimuli with 20 blocks of five consecutive standards randomly intermingled (interstimulus interval (ISI): 0.5 sec, interblock interval (IBI): 0.8 sec), and (2) Dual: 80 blocks of one standard immediately followed by one deviant (ISI: 0.5 sec, IBI: 1-6 sec). Standards were pneumatic tactile stimulation on right index fingertip while in deviants, tactile stimulation was applied to the right forefinger distal and middle phalanges. Ten adults also underwent: (1) Oddball_long_IBI: similar to Oddball but with longer and variable IBI, randomly set between 1-2.5 sec, (2) Expected_Omissions: 100 blocks of four standards followed by an omission with 20 blocks of five consecutive standards randomly intermingled and (3) Rare_Omissions: 180 blocks of five consecutive standards with 35 blocks of four standards followed by one omission randomly intermingled. Non-parametric clustering tests were used for statistics in sensor space. The sMMN generators were localized using conventional equivalent current dipole modeling. Paired t-tests were used to compare the maximal amplitude of source waveforms. In all conditions, significant mismatch responses were elicited at contralateral SII between 75-125 ms post-deviants/omissions. In oddball paradigms, deviants elicited higher SII response than standards whereas in Dual, deviants led to smaller SII response than standards. SII responses to standards in Oddball were similar contrasting with SII response suppression from the second standard in Oddball_long_IBI. No significant SEF difference was observed between standards and omissions but rare omissions led to significantly higher SII responses than expected omissions. These results fit the predictive coding framework. Indeed, at SII cortex, response suppression is observed after a first tactile stimulation, omissions lead to responses similar to those observed after standard stimulation and rare omissions elicit higher responses than expected omissions. Furthermore, response suppression suggests that after a first tactile stimulation, top-down predictions of potential upcoming somatosensory stimuli are dominated by predictions on sensory modality, while after several similar tactile stimuli, the memory trace of tactile stimuli specific features is the main guide of top-down predictions.

Disclosures: G. Naeije: None. V. Wens: None. B. Marty: None. T. Vaulet: None. M. op de Beeck: None. S. Goldman: None. X. De Tiège: None.

Poster
515. Somatosensory Cortex

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 515.12/Q8

Topic: F.01. Human Cognition and Behavior

Support: DFG Graduate Program: Function of Attention in Cognition

Title: Prestimulus oscillatory state differentially influences cerebral processing of attended versus unattended somatosensory stimuli

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Abstract: Background oscillations have been shown to influence the perceptibility of weak attended and unattended stimuli as well as mid-latency ERP components such as the N1. We have recently shown that subthreshold somatosensory stimuli are associated with an evoked potential after 60ms (P1, Nierhaus et al., 2015) which is modulated by selective attention. Here we investigate whether background Rolandic alpha rhythm modulates P1 and - if so - whether this modulation depends on attention, both for subthreshold as well as for suprathreshold stimuli. Subjects (n=40, average age=25 years, 20 females), received subthreshold (imperceptible) and rare suprathreshold (perceptible) electrical pulses over 26 two-minutes blocks to the left index finger while 32 channel EEG was recorded. Subjects were instructed to respond to perceived stimuli only at the cued side. The stimulus locked somatosensory evoked potential (P1 component, SEP) was related to power binned pre-stimulus Rolandic alpha. As expected attention strongly modulated overall Rolandic alpha power, with higher amplitudes contralateral to the unattended side. For attended imperceptible as well as perceptible stimuli, there was a positive relationship between pre-stimulus background Rolandic alpha power and the amplitude of the P1. A different pattern was seen for unattended stimuli: Pre-stimulus Rolandic alpha power was negatively associated with P1 amplitude for unattended suprathreshold stimuli and no clear relationship was seen for unattended imperceptible stimuli. The influence of prestimulus Rolandic alpha power on the SEP seems to differentially depend on the presence of attention both for subthreshold as well as suprathreshold stimuli. Since attention itself modulates baseline alpha, this effect is reminiscent of previous findings reporting an inverse U-shaped relationship between sensory perception and the amplitude of prestimulus alpha (Linkenkaer-Hansen et al., 2004; Zhang & Ding, 2009). Alternatively, the effect might be mediated by a third, but so far
unknown, mechanism, changing the functional role of prestimulus alpha according to the current attentional state.

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**Poster**

515. Somatosensory Cortex

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 515.13/Q9

**Topic:** D.09. Tactile/Somatosensory Systems

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- UZH Forschungskredit 541541808
- NIH BRAIN 1U01NS090475-01

**Title:** Simultaneous calcium imaging of identified feedforward and feedback cortico-cortical neurons during behavior

**Authors:** J. L. CHEN¹, F. F. VOIGT¹, R. KRÜPPEL², *F. HELMCHEN¹;
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- ²Deutsche Forschungsgemeinschaft (DFG), Bonn, Germany

**Abstract:** Cortico-cortical processing is essential for neocortical function during behavior. Understanding the dynamics in information flow across cortex requires simultaneous functional measurements across distant areas. However, it has not been possible to restrict such recordings to neuronal subpopulations that anatomically project between areas. Here, we report the application of a multi-area two-photon microscope in combination with anatomical tracers for simultaneous calcium imaging from identified neurons in distant cortical regions. We used virus-mediated conditional expression of fluorescent reporters, in primary (S1) and secondary (S2) somatosensory whisker cortex to label subsets of "feedforward" S1 neurons projecting to S2 (S1FF) and "feedback" S2 neurons to projecting S1 (S2FB) in layer 2/3 neurons along with the genetically-encoded calcium indicator, YCNano140. Simultaneous calcium imaging was
performed across the corresponding whisker barrel column in S1 and S2 of mice (n = 7 mice, ~1000 neurons per area) engaged in a texture discrimination task or during passive texture presentation. Using linear discriminant analysis and pair-wise correlation analysis, we identify task-dependent dynamics between S1FF and S2FB neurons evolving during the trial period and differing across trial conditions that correlated with texture coding and behavior responses. Our results indicate anatomically-specific coordination of cortico-cortical dynamics under behavior.

**Disclosures:** J.L. Chen: None. F.F. Voigt: None. R. Krüppel: None. F. Helmchen: None.

**Poster**

**515. Somatosensory Cortex**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 515.14/Q10

**Topic:** D.09. Tactile/Somatosensory Systems

**Support:** KAKENHI No. 26460695

**Title:** Sevoflurane modifies information transfer across the cerebral cortex

**Authors:** *J. KURATA;
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**Abstract:** Introduction: Peripheral sensory information reaches the cerebral cortex and induces sensory evoked potentials even under general anesthesia. General anesthesia might possibly induce loss of consciousness by inhibiting sensory information integration at the cerebral cortex resulting in the failed perception of sensation. Propofol and midazolam suppressed activity at the parietal sensory association cortex and transcortical information transfer, respectively. To obtain direct evidence of transcortical suppression, we examined electrocorticogram and corticocortical evoked potentials by grid electrodes under general anesthesia. Methods: Three adult patients underwent implantation of cortical grid electrodes to localize epileptic foci. Several cortical grids were placed on the cortical surface over suspected epileptic foci under sevoflurane anesthesia. End-tidal sevoflurane concentration was kept at 1.5%, sedation evaluated with modified observer's assessment of alertness/sedation scale (mOAA/S); and somatosensory (SSEP), corticocortical evoked potentials (CCEP), and spontaneous electrocorticogram (ECoG) were recorded. The same observation and recording were repeated at 1.0%, 0.7%, and 0% of sevoflurane concentration. Results: The patients regained consciousness at 0.7% or 0% of sevoflurane with the mOAA/S scores of 4-5. The CCEP, but not SSEP, amplitude increased remarkably on awakening (Figure). The ECoG showed the minimum cross-electrode coherence.
and phase reversal at 1% of sevoflurane. Conclusion: Sevoflurane affected the corticocortical information transfer as evidenced by the CCEP and ECoG coherence. Sevoflurane-induced unconsciousness could possibly involve suppression of corticocortical information transfer.

Disclosures: J. Kurata: None.

Poster

515. Somatosensory Cortex

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 515.15/Q11

Topic: D.09. Tactile/Somatosensory Systems

Support: NIH Grant R01GM111293

Title: Disrupted sensorimotor communication during ketamine anesthesia

Authors: *K. E. SCHROEDER*¹, Z. T. IRWIN², M. GAIDICA², J. BENTLEY⁴, P. G. PATIL⁵,⁶, G. A. MASHOUR²,³,⁶, C. A. CHESTEK¹,²,³; ¹Biomed. Engin., ²Neurosci. Grad. Program, ³Ctr. for Consciousness Sci., Univ. of Michigan, Ann Arbor, MI; ⁴Neurosurg., ⁵Neurol., ⁶Anesthesiol., Univ. of Michigan Med. Sch., Ann Arbor, MI

Abstract: The neural mechanisms of anesthetic-induced unconsciousness have yet to be fully elucidated. Most clinically-used general anesthetics act by potentiating the transmission of γ-aminobutyric acid (GABA), which would predictably lead to depression of neuronal function and conscious processing. Ketamine, however, does not depress the cortex and fails to conform to most mechanistic frameworks of general anesthesia: it does not bind with high affinity to the GABAA receptor or depress thalamic metabolism, among other differences. Identifying common neural features of ketamine and GABAergic anesthetics would therefore be an important step
toward a foundational understanding of anesthetic-induced unconsciousness. During the waking state, sensory representation in primary somatosensory cortex (S1) is shared directly with primary motor cortex (M1); they are anatomically and functionally connected. We recorded and analyzed multi-unit thresholded activity, as well as single unit activity, from M1 and S1 of two monkeys while applying sensory stimulation to the finger pads before, during, and after ketamine anesthesia. Before ketamine administration, the identity of the stimulated finger (1 of 3 choice) could be correctly classified from thresholded neural activity at the M1 and S1 electrodes using a Naïve Bayes decoder with a mean accuracy of 74.9% from M1 electrodes and 83.5% from S1 electrodes. During ketamine-induced unconsciousness, however, decoding performance in M1 decreased to chance levels, with a mean of 27.5% correct, while S1 decodes remained unchanged. Furthermore, single-unit firing rate correlations decreased significantly (p<.05) between M1 and S1 cells in the anesthetized state, indicating a functional breakdown in communication. Given past studies showing that GABAergic drugs inhibit surrogate measures of cortical communication, this finding suggests functional disconnection of cortical areas represents a common network-level mechanism of anesthetic-induced unconsciousness.

system. Recent studies have shown that in the tactile space, represented by a spatial-frequency parameter space, the frequency is preserved within the S-M system, particularly in the low frequency range (10 - 50 Hz), but the frequency response properties within the S-M system are not fully described. Here, we investigated the natural and driven oscillation properties and resonance characteristics of S-M system, and compared the following responses within the cortex. The tactile stimulation was given to the forepaw of the lightly anesthetized mice, and the optogenetic stimulation was applied to the forelimb region of the primary somatosensory cortex of freely moving animal to mimic the cortical response to the rhythmic tactile perception. Extracranial high density electroencephalogram (hdEEG) was measured and compared at various frequencies ranging 1 - 60 Hz, and the paw movement was analyzed using paw tracker. Our main findings are (i) the cortical regions corresponding to primary somatosensory and motor cortex responded to paw and cortical stimulation with their maximal responses at beta band frequency (~ 30 Hz) in both cases, (ii) the forepaw motion exhibited resonance response as well but at lower frequency range (~ 20 Hz). These results indicate that the frequency resonance properties are well preserved to the primary somatosensory cortex, but the frequency is down-shifted in signal transduction from the primary somatosensory to muscle. We will discuss the potential role of resonant oscillations in the tactile exploration and potential reason of the frequency downshift in the S-M system.

Disclosures: D. Lee: None. J. Choi: None.
Abstract: A mutation in casein kinase 1delta (CK1d) was identified in two families with advanced sleep phase syndrome and typical migraine with aura. Mice genetically engineered to express this mutation showed a reduced threshold for cortical spreading depression (a phenomenon that underlies both migraine aura and brain injury) and an increased sensitivity to nitroglycerine-induced mechanical and thermal hyperalgesia (both considered migraine relevant pain phenotypes). Cellular mechanisms underlying hyperexcitability are unclear. We examined the intrinsic and synaptic properties of layer 2/3 neurons of mouse somatosensory cortex, using in vivo whole-cell current-clamp recordings in wild-type and CK1d mutant mice. Neurons from CK1d mice showed a hyperpolarizing shift in the resting membrane potential and an increase in input resistance, compared to wild-type animals, suggesting enhanced intrinsic membrane properties. To characterize the synaptic activity, we recorded up and down states in layer 2/3 neurons. We showed an increased duration and amplitude with a lower frequency of spontaneous post-synaptic potentials. In conclusion, CK1d animals have altered baseline excitability, consistent with a migraine-relevant phenotype. CK1d mice represent the transgenic model of non-hemiplegic migraine, thus they may be helpful in elucidating mechanisms that are relevant to normal migraineurs. Support: CDMRP 55900428, R01 59311100

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Poster

515. Somatosensory Cortex

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Topic: D.09. Tactile/Somatosensory Systems

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Title: Identifying hand, foot and face sensorimotor U-fibers using DTI

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3Kennedy Krieger Inst., Baltimore, MD; 4Integrated Brain Imaging Ctr., Univ. of Washington,
Seattle, WA

Abstract: Introduction: U-shaped white matter bundles (“U-fibers”) represent short association
fibers connecting adjacent gyri and are critical to cortico-cortical integration. Sensorimotor U-
fibers are comprised of connections between primary somatosensory and motor regions.
Mapping sensorimotor U-fibers and segmenting these fibers into functionally distinct clusters
that correspond with the foot, hand, and face could provide insight into white matter
development and aid in elucidating underlying white matter abnormalities in developmental and
neurodegenerative disorders. Few studies have attempted to segment sensorimotor U-fibers using
diffusion imaging; however, their ability to demonstrate anatomic validity and intra- and inter-
rater reliability is limited. In this study, we leverage fMRI motor execution literature with
diffusion imaging to reconstruct sensorimotor U-fibers corresponding with the foot, hand, and
face and demonstrate a high degree of reliability. Methods: Fiber tracking was performed on 10
participants: 4 autism, 3 ADHD, and 3 controls, aged 8-12 yrs. fMRI-based seeds served as
centroids for spheres used to segment the sensorimotor fiber map into foot, hand, and face
regions (figure 1). Extraneous fibers not connecting sensorimotor cortices were removed. The
procedure was performed by two raters. Intra- and inter-rater reliability was calculated using
Dice’s coefficient. Results: The mean Dice’s coefficients for intra-rater reliability were 0.99,
0.99, and 0.98 for foot, hand, and face, respectively; inter-rater reliability for foot, hand, and face
was 0.98, 0.98, and 0.95, respectively. Conclusions: In this study we combined motor activation
coordinates with DTI fiber tracking and demonstrate feasibility and a high degree of reliability
using this approach. This approach could provide valuable insight into the underlying white
matter microstructure of developmental disorders and further aid in revealing associations
between sensorimotor microstructure and diagnostic and behavioral characteristics of these
disorders.

Poster

515. Somatosensory Cortex

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 515.19/Q15

Topic: D.09. Tactile/Somatosensory Systems

Support: île de France DIM Cerveau&Pensée

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CNRS, Projets Exploratoires Premier Soutien

Title: Optogenetic mapping of translaminar functional connectivity in the somatosensory cortex of rodents

Authors: *M. C. QUIQUEMPOIX*1,2,3, S. L. FAYAD1,2,3, K. BOUTOURLINSKY1,2,3, N. LERESCHE1,2,3, R. C. LAMBERT1,2,3, T. BESSAIH1,2,3,

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Abstract: While the six-layered structure of the neocortex is one of the most prominent features of the mammalian brain, the role of this laminar architecture in information processing is still...
elusive. Multiple simultaneous intracellular recordings as well as glutamate uncaging and laser scanning photostimulation in brain slices allowed the emergence of a picture of the wiring diagram between cortical layers. However, these approaches do not allow evaluating the relationships between the strength of translaminar connectivity and the response properties to sensory stimuli of individual neurons. We used *in utero* electroporation in mice to deliver ChannelRhodopsin-2 to layer 2/3 pyramidal neurons in the barrel cortex of mice. This technique allows the specific, fast and reliable control of the temporal pattern of activity in the transfected neurons, while recording simultaneously the spiking activity of several layer 5 cortical neurons *in vivo*. We found that optogenetic stimulation of layer 2/3 pyramidal neurons enhances the activity of only a subset of layer 5 neurons, which have particular response properties to tactile stimulations.

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**Poster**

**515. Somatosensory Cortex**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 515.20/Q16

**Topic:** D.09. Tactile/Somatosensory Systems

**Support:** International Research Training Group for Schizophrenia and Autism - IRTG 1328, Germany

**Title:** Morphological and Functional Characterization of Non-fast spiking GABAergic Interneurons in layer 4 microcircuitry of rat barrel cortex

**Authors:** *V. SIVARAJAN*¹,², G. QI², D. FELDMEYER²,³; ¹Dept. of Psychiatry, Psychotherapy and Psychosomatics, RWTH Aachen Univ. Hosp., Aachen, Germany; ²Inst. of Neurosci. and Med. - 2, Forschungszentrum, Juelich, Germany; ³Translational Brain Med. (JARA Brain), Jülich Aachen Res. Alliance, Aachen, Germany

**Abstract:** Inhibitory GABAergic interneurons (INs) are notorious for their heterogeneity. Classification of INs is crucial for understanding their functions. GABAergic INs can be broadly classified into fast spiking (FS) and non-fast spiking (nFS) INs based on their intrinsic firing patterns. Recently, the functional and structural properties of nFS INs (e.g. somatostatin-positive INs) have received more attention. In this study, we focused on the quantitative morphological classification of nFS INs in layer 4 (L4) of the rat primary somatosensory (barrel) cortex and
their connectivity profiles. Single and paired whole-cell patch-clamp recordings were performed on acute barrel cortex slices of juvenile rats. Following histochemical processing, biocytin-filled neurons were morphologically reconstructed using the Neurolucida system. Fluorescent dyes conjugated with biocytin were used to investigate the molecular marker expression in patched L4 nFS INs. Unsupervised cluster analysis (Ward’s method) was adopted to classify L4 nFS INs based on their quantitative electrophysiological and morphological parameters. Principal component analysis was used to eliminate the correlated parameters. We identified 4 morphologically distinct L4 nFS IN types. These types differed with respect to their intralaminar, intracolumnar and translaminar axonal projection patterns. The majority of L4 nFS INs were somatostatin-positive. We also found that L4 nFS INs show diverse firing patterns such as late-spike, stuttering and irregular spiking. Results from paired recordings from synaptically coupled nFS INs and other L4 neurons showed that nFS INs establish weak synaptic connections, with a low release probability and low amplitude; the connections showed paired-pulse facilitation. This is markedly different from the FS INs, which form strong synaptic connections that exhibit invariably paired-pulse depression. Based on the above findings, we conclude that nFS INs have very distinct morphological and physiological characteristics compared to FS INs and are an important element in the L4 microcircuitry. Elucidating the structure and function of nFS INs is critical for the understanding of the role of inhibitory microcircuits in health and disease.

Disclosures: V. Sivarajan: None. G. Qi: None. D. Feldmeyer: None.

Poster

515. Somatosensory Cortex

Location: Hall A

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Topic: D.09. Tactile/Somatosensory Systems

Support: NINDS R01 NS069679

Dana Foundation Brain Imaging Program

Title: Behavioral rewards impact sensory cortical representations via their effect on apical dendrites in Layer 1 of mouse barrel cortex

Authors: *C. LACEFIELD1, E. PNEVMATIKAKIS2, L. PANINSKI1,2,3,4, R. M. BRUNO1,5; 1Neurosci., 2Statistics, 3Ctr. for Theoretical Neurosci., 4Grossman Ctr. for the Statistics of Mind,
Abstract: Layer 1 of mammalian sensory cortex is potentially one of the most important sites where sensory signals and internal signals are integrated. In the rodent vibrissal primary somatosensory “barrel” cortex, Layer 5 pyramidal neurons send apical dendrites to the surface of the cortex in Layer 1, where they receive excitatory synaptic contacts from the vibrissal motor cortex (vM1), thalamic medial posterior nucleus (PoM), and secondary somatosensory cortex (S2), as well as a host of contacts from diverse inhibitory interneurons and neuromodulatory nuclei. These connections have the potential to influence the processing of whisker sensory information within the cortical circuit in the context of information about self-generated motion, higher-order features of a stimulus, behavioral state, or the relevance of a sensory stimulus to behavior. To elucidate the impact of these connections onto Layer 1 apical tuft dendrites during behavior, we imaged dendritic calcium activity in a broad population of dendrites from Layer 5 pyramidal neurons in the mouse barrel cortex using two-photon imaging of the genetically encoded fluorescent calcium indicator GCaMP6 while mice performed a whisker-based head-fixed operant task. We find that calcium activity within Layer 1 correlates with timing of rewards given during a stimulus presentation, as well as with random rewards given during non-stimulus epochs. Examining the activity of individual apical tufts reveals that the effect of reward is non-uniform across neurons, rather than a singular broad influence over the entire population. This finding suggests that reward may play a role in modulating subpopulations of neurons that underlie sensation during behavior. These influences could lead to enhancement of sensory representations for behaviorally relevant stimuli, or alternately, for learning associations between stimuli or with behavioral responses necessary for attaining rewards.

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Poster

515. Somatosensory Cortex

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Topic: D.09. Tactile/Somatosensory Systems

Support: CRC 889-TP C 07

DFG Sta 431/8-1
Title: VIP interneurons in the barrel cortex of VIPcre/tdTomato mice

Authors: A. PRÖNNEKE1, B. SCHEUER1, R. J. WAGENER1, M. MOECK1, M. WITTE1, *J. F. STAIGER2;
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Abstract: The vast heterogeneity in the population of neocortical GABAergic interneurons provides a major obstacle in identifying and studying distinct subgroups. This hampered the understanding of their individual properties and functional impact onto cortical circuitry and information processing. Now, distinct and virtually non-overlapping subgroups of inhibitory interneurons can be targeted by the expression of cre under the promoter of certain proteins, like parvalbumin, somatostatin and vasoactive intestinal polypeptide (VIP). Among these, VIP expressing interneurons sparked substantial interest since these neurons seem to operate disinhibitory circuit motifs found in all major neocortical areas. However, only very little is known about neurochemical specificity and electrophysiological as well as morphological properties of neurons tagged in transgenic Vip-ires-cre mice. Therefore, the potential to specifically target the population of VIP expressing interneurons in this mouse line was evaluated. We quantitatively analyzed the co-localization of cre-driven fluorescence with labeling deriving from in-situ hybridization using Vip RNA probes or αVIP immunohistochemistry in the barrel cortex of VIPcre/tdTomato mice in a layer-specific manner. Our results indicate a high sensitivity (complete coverage of the target cell population) and specificity (virtually 100% of co-localization in immunostainings) of cre expression for VIP neurons, and also verified that these are GABAergic interneurons (i.e., Gad1 positive). Furthermore, we determined the distribution pattern of VIP interneurons in the barrel cortex of mice, which are predominantly found in layer (L) II/III (58.7%). However, a substantial fraction (39.4%) is located in L IV to VI. Interestingly, we found differentially distributed morphological features of single VIP neurons from L II/III when compared to L IV-VI. The most conspicuous difference was that the dendritic input domain of L II/III neurons was restricted to L I-III whereas the axonal output was distributed across all layers. In contrast to this, the dendrites of VIP neurons in L IV-VI were significantly more extended vertically, often reaching the upper layers, whereas the axon remained virtually confined to the layers V-VI. Furthermore, VIP neurons displayed a variety of firing patterns, which did not correlate with a broad spectrum of electrophysiological properties or morphological characteristics. VIP neurons in L IV-VI, however, were more hyperpolarized, had a greater amount of fast inward rectification and larger amplitudes of AHPs compared to those in L II/III.

Title: Fast modulatory activity of serotonergic cortical afferents mediated by 5HT3AR-expressing interneurons

Abstract: Serotonin (5-HT) is thought to play a critical role in neuromodulation during specific brain states and behavioral contexts. In the mouse cerebral cortex, 5-HT is released from Raphe Nuclei (RN) afferents and acts on a plethora of excitatory or inhibitory 5-HT receptors expressed on pyramidal (Pyr) neurons as well as on GABAergic interneurons. The vast majority of these receptors are metabotropic. The only ionotropic serotonergic receptor (5HT3AR) is excitatory and is expressed in almost one third of cortical GABAergic interneurons. Thus serotonin may exert fast modulatory effects on and play a pivotal role in shaping cortical activity. To unravel the role of 5HT3AR interneurons in serotonergic modulation in cortical microcircuits, we performed targeted patch recordings in the barrel cortex, in acute brain slices of transgenic mice in which 5HT3AR interneuron population or its vasoactive intestinal peptide (VIP)-expressing subset is fluorescently labeled. Combining optogenetics with local electrical stimulation and pharmacology, we characterized serotonergic inputs to 5HT3AR interneurons and explored the modulatory roles of these inputs as well as of VIP-expressing interneurons in cortical microcircuits. Local application of the potent high affinity 5HT3AR agonist 1-(m-chlorophenyl)-biguanide (mCPBG) elicited excitatory post-synaptic potentials (EPSPs) and spiking activity in both VIP-positive and VIP-negative 5HT3AR interneurons confirming the depolarizing action of 5HT3ARs in those cells. Moreover, optogenetic stimulation of RN cortical afferents elicited excitatory responses in a significant fraction of the 5HT3AR-GFP interneurons. These responses
persisted in the presence of glutamatergic receptor blockers, providing evidence for direct serotonergic depolarization of these cells via synaptic transmission. Simultaneous optogenetic stimulation of RN afferents and electrical stimulation of layer 4 (L4) elicited greatly different responses in Pyr, somatostatin (SOM)-expressing and parvalbumin (PV)-expressing neurons, suggesting that serotonin release has distinct short-latency effects on those neurons. Interestingly, similarly distinct responses were observed upon combined optogenetic stimulation of VIP-expressing neurons and electrical stimulation of L4. Altogether, our work provides evidence that serotonin release from RN afferents in the barrel cortex recruits 5HT3AR interneurons, affecting synaptic inputs to principal neurons and other GABAergic interneurons.

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Poster

515. Somatosensory Cortex

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 515.24/Q20

Topic: D.09. Tactile/Somatosensory Systems

Support: PSC CUNY

Title: The effect of sensory deprivation and the role of perineuronal nets

Authors: P. CHU¹, K. BUDHU², *J. C. BRUMBERG³;  
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Abstract: Perineuronal nets (PNNs) are specialized extracellular matrix structures of the central nervous system that predominantly ensheathe inhibitory interneurons. The development of PNNs is activity dependent relies on sensory input to mature to normal levels and is correlated with the closure of the developmental critical period. It remains unknown how PNNs affect electrophysiological intrinsic properties of neurons and how that may influence sensory deprivation induced plasticity. After 30 days of global bilateral sensory deprivation of the barrel cortex we found prolonged reductions in PNNs density in the somatosensory barrel cortex. Even after a period of sensory recovery for another 22 days following deprivation, we do not see any recovery off PNN levels. Our results provide support for the idea of a developmental critical period for PNN development. In vitro whole cell patch clamp recordings from barrel cortex following one month of deprivation resulted in changes in the response properties of fast-spiking
interneurons consistent with the known cellular distribution of PNNs. To further study the role PNNs have in modulating intrinsic and synaptic properties of barrel cortex neurons we used an in vitro model of PNN reduction via enzymatic digestion by Chondroitinase ABC (chABC) in adult mice, followed by whole cell patch clamp recordings. We found that intrinsic physiology of PNN regulation of GABAergic inhibitory cells i.e., Fast spiking and Low threshold spiking cells, was impacted to a greater extent than that of excitatory neurons i.e., Regular spiking, Regular spiking doublet, and Bursting cells. Regular spiking-Single and Regular spiking-doublet cells were not found to be greatly impacted by PNN digestion. These findings suggest that PNNs are critical regulators of intrinsic physiological plasticity in a cell type specific manner.

Disclosures: P. Chu: A. Employment/Salary (full or part-time); Queens College, CUNY. K. Budhu: None. J.C. Brumberg: A. Employment/Salary (full or part-time); Queens College, CUNY.

Poster

515. Somatosensory Cortex

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 515.25/R1

Topic: D.09. Tactile/Somatosensory Systems

Support: DFG Barrel Cortex Function (BaCoFun)

Title: Inter-barrel synaptic connections involving layer 4 spiny neurons and interneurons in rat barrel cortex

Authors: *G. Qi¹, D. Feldmeier¹,²;
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Abstract: In the primary somatosensory (barrel) cortex of rodents, the neuronal network in a single layer 4 (L4) barrel has been considered to be a self-contained entity with little interaction with adjacent barrels; this is significantly different from all other cortical layers where long-range horizontal synaptic interactions are known to exist. In the present study we present direct evidence for the existence of monosynaptic connections between L4 neurons located in two separate barrels and characterise their electrophysiological and morphological properties using paired recordings and simultaneous biocytin fillings. Monosynaptic connections (n=18) between L4 neurons located in two neighbouring barrels were obtained. They include 8 excitatory-excitatory (E-E), 8 excitatory-inhibitory (E-I) and 2 inhibitory-excitatory (I-E) connections. Once a postsynaptic neuron was patched in one barrel, normally less than 10 (1 - 12) putative
presynaptic neurons located in the adjacent barrel need to be searched before one connection was found. The distance between the somata of pre- and postsynaptic neurons is in the range of 126 - 272 µm. E-E connections (n=8) including two reciprocal ones have an average unitary EPSP (uEPSP) amplitude of 0.71 ± 0.50 mV, coefficient of variation (CV) of 0.42 ± 0.21 and failure rate (FR) of 22.3 ± 25.2%. The amplitude was depressing in most (6 out of 8) connections during repetitive presynaptic suprathreshold stimulations at 10 Hz. The 20-80% rise time and decay time constant are 1.59 ± 0.48 ms and 33.2 ± 15.0 ms, respectively. All of the aforementioned parameters for inter-barrel connections are not statistically different from that of intra-barrel ones. However, the latency of 1.71 ± 0.21 ms is significantly longer than that of intra-barrel ones (~1 ms). There is no preference for two L4 spiny neuron subtypes, i.e., spiny stellate cell (SSC) and star pyramidal neuron (SPN), to be pre- (5 SSCs vs. 3 SPNs) or postsynaptic (4 SSCs vs. 4 SPNs) neurons. E-I connections (n=8) have an average uEPSP amplitude of 0.70 ± 1.13 mV, CV of 0.56 ± 0.21 and FR of 25.3 ± 23.0%. They are much weaker than intra-barrel connections (≥1 mV). The uEPSP amplitude was either depressing or weakly facilitating with a paired-pulse ratio of 0.96 ± 0.19. The rise and decay time are 0.53 ± 0.22 ms and 9.9 ± 3.1 ms, respectively. The latency is 1.92 ± 0.83 ms, which is significantly longer than that of intra-barrel ones (~0.5 ms). Pre- and postsynaptic neurons are predominantly SSCs (7 out of 8) and fast spiking (FS) interneurons (7 out of 8), respectively. In addition, two monosynaptic inhibitory synaptic connections from FS interneurons in one barrel to SSCs in the other barrel were recorded.

Disclosures: G. Qi: None. D. Feldmeyer: None.

Poster

515. Somatosensory Cortex

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 515.26/R2

Topic: D.09. Tactile/Somatosensory Systems

Support: MEXT/JSPS KAKENHI 24500408

MEXT/JSPS KAKENHI 25123709

MEXT/JSPS KAKENHI 15K14333

MEXT/JSPS KAKENHI 15H01430

MEXT/JSPS KAKENHI 1301992

MEXT/JSPS KAKENHI 26118508
Title: Excitatory and inhibitory inputs to vasoactive intestinal polypeptide-expressing neurons in the mouse barrel cortex

Authors: *J. SOHN*¹, S. OKAMOTO¹, N. KATAOKA², K. NAKAMURA²,³, T. KANEKO¹, H. HIOKI¹; ¹Kyoto Univ. Grad. Sch. of Med., Kyoto, Japan; ²Dept. of Physiol. 2, Nagoya Univ. Grad. Sch. of Med., Nagoya, Japan; ³PRESTO, JST, Nagoya, Japan

Abstract: Vasoactive intestinal polypeptide-positive (VIP+) neurons, morphologically characterized as bipolar cells, are one of the subclasses in γ-aminobutyric acid (GABA)-ergic inhibitory neurons in the neocortex. Recently, excitation of VIP+ neurons has been revealed to trigger the local cortical activation via inhibition of other types of inhibitory neurons. The presynaptic inputs to VIP+ neurons are, therefore, responsible for the activated state of local cortical network. We morphologically investigated synaptic convergence to the subcellular compartments of VIP+ neurons in layer (L) 2/3 of the mouse barrel cortex. We developed a new adeno-associated virus (AAV) vector; this AAV vector forced neurons to strongly express reporter protein. Injection of AAV2/1-SynTetOff-DIO-FGL into the barrel cortex of VIP-Cre mice resulted in specific labeling of local VIP+ neurons with somato-dendritic membrane-targeted green fluorescent protein (FGL). Reconstruction imaging of VIP+ neuron dendrites showed their bipolarity in the vertical orientation, and their apical branches were longer and ramified at more distal portion than basal branches. We subsequently investigated input patterns to VIP+ neurons; both cortico- and thalamocortical excitatory inputs were frequently found on the distal dendrites of VIP+ neurons, and corticocortical excitatory inputs were nearly two-fold denser than thalamocortical inputs. These dense excitatory inputs on the distal dendrites were not just because of the layer-specific distribution of presynaptic axon terminals, but excitatory inputs preferentially targeted the distal dendrites. Since their ‘apical branches’ abundantly ramified at the distal portion in L1, VIP+ neurons can be strongly recruited by integrative excitatory projections from other neocortical areas and paralemniscal thalamic nuclei. On the other hand, inhibitory inputs were almost equally distributed on the somato-dendritic domain of VIP+ neurons. We finally evaluated the axonal arborization of L2/3 VIP+ neurons; their axons arborized transaminarly from L1 to L6, running in L4 preferentially through interbarrel ‘septa’. Those input-output characteristics may be inherent in VIP+ neurons for their transaminar activation of local cortical networks.


Poster

515. Somatosensory Cortex
Abstract: Movement activates peripheral mechanosensors, giving rise to ‘reafferent’ signals, whereas contacts with objects produce ‘exafferent’ signals. Reafferent and exafferent signals are often multiplexed in the same pathway. The brain needs to disentangle these different streams of information for perception. Movement itself can sculpt tactile signals in the sensory cortex, extracting task-relevant signal at the expense of extraneous signals. The locus and mechanisms of this sensory gating are not fully understood. We investigated the circuit mechanisms of tactile gating in mice performing an object localization task with their whiskers. Neurons in the ventral posteromedial nucleus of thalamus (VPM) are sensitive to both whisker movement and touch. Long-range input from VPM targets primarily layer 4. VPM afferents synapse onto both excitatory neurons (L4_e) and fast-spiking GABAergic interneurons (L4_fs), which in turn inhibit the excitatory neurons. In behaving mice, L4_e neurons responded selectively to touch with temporally sharp, large-amplitude membrane depolarization and spikes, whereas whisker movements by themselves produced little membrane potential change and increase in spike rate. In contrast, L4_fs interneurons responded strongly to touch and whisker movements, similar to VPM neurons. Silencing VPM or cutting the infraorbital nerve abolished whisking-correlated activity in L4_fs neurons, indicating that the inhibition is driven by sensory input via the thalamus. Suppressing activity in L4_fs neurons using eNpHR3.0 revealed whisking-related excitation and enhanced touch responses in L4_e neurons. Thus, fast-spiking interneurons cancel movement-related input in cortical excitatory neurons to extract a sparse and selective representation of touch in the somatosensory cortex.

Title: Laminar specific membrane potential dynamics of forepaw primary somatosensory cortical neurons during behavior

Authors: *W.-J. ZHAO1,2, J. KREMKOW1,2,3, J. F. A. POULET1,2;
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Abstract: Sensory cortex integrates external sensory input with internally generated spontaneous activity across 6 layers of interconnected neurons. The synaptic and cellular mechanisms of trans-laminar sensory integration in behaving animals, however, are poorly understood. Here we made single and dual whole-cell recordings in primary somatosensory forepaw cortex of behaving mice to compare the cellular properties and membrane potential dynamics of L2/3 and L5 excitatory neurons. We show that L2/3 and L5 neurons have distinct intrinsic properties with L5 neurons having larger input resistance and higher firing rates than L2/3 neurons during current injection. Higher firing rates of L5 neurons were also present during spontaneous activity during quiet resting periods, during active periods of forepaw movement and in response to sensory stimulation. We show that these differences in firing rates were signaled by a smaller difference between the peak spontaneous Vm or sensory-evoked reversal potential and action potential threshold in L5 neurons as compared to L2/3 neurons. Dual, simultaneous recordings allowed a direct comparison of the timing of synaptic input and action potential firing across layers. Slow depolarizing events during quiet states were broadly correlated across layers but were more reliable and had an earlier onset in L5. In contrast, synaptic input triggered by movement onset or sensory stimulation had similar latencies and was highly correlated across layers. Movement and sensory evoked subthreshold input therefore showed a different temporal structure across layers compared to spontaneous activity. We conclude that laminar specific
membrane potential properties are central to spike rate differences recorded across cortical layers during active sensory processing.

**Disclosures:**  
W. Zhao: None. J. Kremkow: None. J.F.A. Poulet: None.

**Poster**  
516. Somatosensory Response Properties  
**Location:** Hall A  
**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM  
**Program#/Poster#: 516.01/R5  
**Topic:** D.09. Tactile/Somatosensory Systems  
**Support:** KAKENHI 21650095  
KAKENHI 24700397  
**Title:** Effects of excitation wave induced by forelimb stimulation on the propagation pattern of excitation wave induced by hindlimb stimulation in the rat sensorimotor cortex recorded with an optical recording system  
**Authors:** *N. HAMA, M. KAWAI, S.-I. ITO, A. HIROTA; Shimane University, Sch. of Med., Izumo, Japan  
**Abstract:** Optical recording system using a voltage-sensitive dye has well documented that the sensory-evoked cortical response is of a concentric propagating wave emerging from the focal site rather than a simultaneous activation of the area of response. Thus, when two distant cortical sites are concurrently activated, the two waves should interact with each other. Details of this interaction, however, remain largely unknown. In this study, we examined the influence of forelimb stimulation-induced excitation wave (wave F) on the hindlimb stimulation-induced one (wave H). The sensorimotor cortex was exposed and stained with a dye (RH-414). Electrical stimulation (1 mA, 0.5 ms) was given first to the hindlimb then to the forelimb with an interstimulus interval (ISI) of 0, 10 or 20 ms. We compared the latency of wave H at the initiation site with and without forelimb stimulation. Without forelimb stimulation, the first response to the hindlimb stimulation occurred with the latency of 46.5 ± 10.2 ms (mean ± SD). With forelimb stimulation the latency of wave H was significantly shortened for the ISI of 0 ms. The wave F arrived at the wave H initiation site, 57.0 ± 7.83 ms after the forelimb stimulation. It should be noted that this value was longer than the value of shortened latencies of wave H. We also compared the amplitude, full width at half maximum (FWHM) and slope of the rising phase of wave H with and without forelimb stimulation, at the collision portion of two propagation
waves. To estimate the portion, we constructed the isochrone maps based on the difference in latency of each pixel. And then, the collision portion was defined as the recording portion including the local maximum of the isochrone map within the area between forelimb- and hindlimb-response initiation sites. The FWHM was significantly shorter for all ISIs. The slope of rising phase was significantly steeper but only for the ISI of 0 ms. No significant difference was found in the amplitude for any ISIs. We conclude that this interaction is not linear summation and that the spatio-temporal pattern of neural activity as resulting from the interaction between two waves in the sensorimotor cortex is complex depending on the change of the timing of another sensory input.

**Disclosures:** N. Hama: None. M. Kawai: None. S. Ito: None. A. Hirota: None.

**Poster**

**516. Somatosensory Response Properties**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 516.02/R6

**Topic:** D.09. Tactile/Somatosensory Systems

**Support:** NIH R01NS072416

NSF GSRF to KL

**Title:** Representation of two-whisker sequences in L2/3 of mouse S1

**Authors:** *K. J. LABOY-JUAREZ*, D. E. FELDMAN;

1Univ. of California, Berkeley, Berkeley, CA; 2Helen Wills Neurosci. Inst., UC Berkeley, Berkeley, CA

**Abstract:** Most studies of sensory coding in rodent whisker somatosensory cortex (S1) have focused on understanding the encoding of single whisker stimuli. However, natural whisker sensation involves complex spatiotemporal patterns of whisker contacts, suggesting that multi-whisker stimuli are processed and represented in S1. Here we tested how S1 represents two-whisker combinations, which are the simplest multi-whisker feature. We presented single-whisker stimuli and two-whisker sequences in urethane-anesthetized mice, while performing single unit extracellular recording in layers 2-4 of the D1 column of S1. We interleaved single whisker deflections of a 3x3 grid of whiskers centered on D1 plus all possible two-whisker sequences at ±2 ms intervals (72 sequences). Optimal sequences evoked higher firing rates and response probabilities than optimal single whisker deflections. For 85% of units, optimal
combinations involved adjacent whiskers with a clear preference for sequences involving D1 (columnar whisker) or their best whisker (BW, the whisker that evoked the most spikes). Selectivity among 2-whisker sequences (measured by lifetime sparseness) was higher than predicted from the linear sum of single whisker responses. Selectivity was greater in L2/3 than in L4. L2 units responded linearly to the best sequence but sublinearly to other sequences. L4 units responded sublinearly to all sequences, with non-optimal sequences often being strongly suppressed. Thus, sublinear integration of single whisker input sharpens tuning for 2-whisker sequences. Whisker map topography differed for single-whisker stimuli vs. 2-whisker combinations. Mapped with single-whisker stimuli, the large majority of L4 neurons were tuned to the CW for their column (D1), but L2/3 neurons exhibited heterogeneous tuning for multiple whiskers. This is consistent with a recent mapping study using calcium imaging (Clancy et al., 2015). However, when mapped using 2-whisker sequences, many L2/3 neurons responded best to sequences involving the D1 whisker. Further, some L2 single units that were not tuned to D1 when mapped with single whisker deflections responded more strongly to D1 than any other whisker when 2-whisker sequences were presented. These initial results suggest that many L2/3 neurons are tuned for specific 2-whisker sequences, and that multi-whisker representation may increase between L4 and L2/3. We speculate that a major role of S1 circuits may be to process multi-whisker information.


Poster

516. Somatosensory Response Properties

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 516.03/R7

Topic: D.09. Tactile/Somatosenosory Systems

Support: INSERM

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   FRC

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Title: Sensory stimulus evoked responses in layer 2/3 pyramidal neurons of the hind paw-related mouse primary somatosensory cortex
Abstract: The mouse primary somatosensory cortex (S1) processes tactile sensory information and is, with a surface area of ~30%, the largest sensory neocortex area, emphasizing the importance of this sensory modality for rodent behavior. Most of our knowledge regarding information processing in S1 stems from studies of the whisker-related barrel cortex (S1-BC), yet the processing of tactile inputs from the paws is largely unknown. We used whole-cell patch-clamp recordings from layer (L) 2/3 pyramidal neurons of the S1 hind-paw (HP) region of anaesthetized mice to investigate their intrinsic properties, spontaneous activity, and evoked sub- and supra-threshold activity. We found that L2/3 neurons displayed up- and down-states characteristic of sleeping mice, and low spontaneous firing rates (~0.1 Hz). Approximately 45% of L2/3 pyramidal neurons responded to contralateral HP stimulation in a sub-threshold manner, ~5% with on average 0.4 action potentials per trial, while ~50% of neurons did not respond at all. The evoked subthreshold responses had a long onset (23 ms) and peak latency (61 ms), and 86% of L2/3 neurons responded to prolonged stimulation with on- and off-responses. HP stimulation responsive neurons had a greater intrinsic excitability compared to non-responding neurons, as shown by an increase in the action potential output as a function of current injected. In conclusion, while S1-HP L2/3 pyramidal neurons share certain physiological properties with their counterparts in the S1-BC, they also have unique features specific for the sense of paw touch. Our findings support a sparse coding scheme of operation for S1-HP L2/3 pyramidal neurons, and suggest that response latencies are not crucial for paw sensation and sensory aspect of locomotion.

Disclosures: G. Bony: None. A. A-Bhaskaran: None. K. Le Corf: None. A. Frick: None.
Abstract: The whisker barrel system functions as a sensory-motor loop, with tight interconnections between the sensory S1 barrel cortex and the vM1 motor cortex. During physiological whisking, neurons in the barrel cortex receive information regarding whisker movements from vM1 followed by upstream sensory information arriving from the whiskers via the thalamus. The goal of this study is to investigate the effect of motor inputs arriving from vM1 on sensory processing in the S1 barrel cortex. Under physiological conditions the activity in the vM1 motor and sensory barrel cortices are tightly linked. To dissociate between the activity in these two cortices we stimulated whiskers either passively (ramp and hold stimulation) or with artificial whisking paradigms (facial nerve stimulation induced artificial whisking against P320 sandpaper), and independently activated the motor cortex using channel rhodopsin 2 (ChR2) mediated optogenetic stimulation. We monitored the response in S1 barrel cortex neurons using a 16-contact single shaft silicon probe that simultaneously recorded single units from layers 2-5. Pairing optogenetic activation of VM1 (20 ms laser pulse) with whisker activation (either passive or artificial whisking) yielded a prominent supra-linear summation of the two responses. On average the response of paired optogenetic vM1 activation with passive whisker deflection and artificial whisking against sand paper was 178±49 % and 168±56 % of the expected for linear summation of the two responses (P< 0.005). This supra-linear summation response was largest in layer 5 neurons and smallest in layer-4 neurons fitting previously known functional neuroanatomical data about vM1-S1 connectivity. When we changed the relative timing of optogenetic stimulation and whisker activation we found that the response of both passive whisker stimulation and artificial whisking activation can be enhanced supra-linearly when vM1 was optogenetically activated 75 ms before up to 20 ms after the initiation of whisker stimulation. In these experiments, maximal supra-linear enhancement was achieved when vM1 was activated 20 ms before whisker activation (both for passive and artificial whisking paradigms). In conclusion, our findings suggest that as whisking is initiated, vM1 sends parallel "attentional" information to S1 barrel neurons, preparing them for the sensory cues expected to arrive from the periphery.

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Poster

516. Somatosensory Response Properties

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 516.05/R9

Topic: D.09. Tactile/Somatosensory Systems

Support: DFG CH1232/1-1
Title: Sensory modulation by whisker movement in the rat brainstem trigeminal complex

Authors: S. CHAKRABARTI, *C. SCHWARZ;
Univ. Tuebingen, Tuebingen, Germany

Abstract: In order to make sense of input acquired by mobile sensors, information about movement and sensory input needs to be integrated. In the rodent whisker system this interaction happens as early as in the brainstem trigeminal nuclei (TN). The TN house the second order tactile neurons, but also a quite extensive and only partially known intrinsic network. Lesions in one part of the TN, the ncl. spinalis interpolaris caudalis (SpVic) lead to blockade of movement effects in the lemniscal pathway ascending from the principal nucleus (PrV). We aimed at elucidating the hypothesis that SpVic is the origin of motor effects in PrV by recording unit and LFP data in both sub-nuclei in head-fixed rats trained to perform active whisker movements to touch an object (Schwarz et al., 2010, Som. & Mot. Res., 27:131). The animals were implanted with mobile multielectrode arrays to record unit and evoked LFP data in PrV and SpVic. We find that during active whisker movements both nuclei show a depression of the sensory response to object contact with concomitant increment of (pre-touch) movement-related activity. The majority of neurons in both the PrV and SpVic showed a bimodal response to touch, perhaps indicating direct input from primary afferents and an indirect one from the well-described corticobulbar projections. The identical modulation of the two sub-nuclei in unit and LFP data does not support the notion that SpVic is responsible for sensory modulation in the lemniscal pathway. Currently we aim at identifying the intermingled ascending projection neurons and intrinsic neurons in SpVic to gain more detailed insight into SpVic's role for active touch.

Disclosures: S. Chakrabarti: None. C. Schwarz: None.

Poster

516. Somatosensory Response Properties

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Topic: D.09. Tactile/Somatosensory Systems

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NSF GRFP DGE-1148897

Title: Receptive fields and response characteristics of neurons in the S1 whisker representation of the short-tailed opossum, Monodelphis domestica
Abstract: The innovation of hair in mammalian evolution allowed for the development of a unique form of movable tactile sensors in the form of sinus hairs, or whiskers. Whiskers are present in all marsupials and placental mammals. However, to date, neural response properties in the cortical whisker representation have been studied in only two species of rodents (rats and mice), and to a small extent in lagomorphs (rabbits). Using single unit extracellular recording, we examined the receptive fields and response properties of neurons in the whisker representation of the short-tailed opossum, a marsupial that shared its last common ancestor with placental mammals over 160 million years ago. Single neurons in layer IV of the S1 whisker representation in the opossum predominantly respond to a single best whisker. In addition, these neurons also display a lower magnitude response to 1-15 surrounding whiskers. Thus, opossum S1 neurons have a receptive field organization with a center and an excitatory surround, similar to the neurons in S1 barrel cortex of rodents. Peristimulus time histograms indicate that S1 neurons display a transient response to stimulus onset and offset. In general, the offset response has a lower magnitude compared to the onset response. On average, neurons respond to stimulus onset with a short latency of 5-10 ms, similar to neurons in S1 barrel cortex of rodents. The population mean response profiles were examined for neural responses to the best whisker, compared to the surrounding whiskers, and were found to have similar response latencies. The short latency in the neural responses to the best whisker as well as the surrounding whiskers suggests the contribution of direct thalamocortical inputs to the excitatory surround component of the receptive fields of neurons in the opossum S1 whisker representation. In rodents, the receptive field surround is mainly driven by intracortical relays rather than direct thalamocortical input. The distribution of spike waveform durations in opossum S1 was bimodal, with an average spike duration of 0.3 ms for one set of neurons, and 0.6 ms for the other. This is similar to previous findings in somatosensory cortex of mice, rats and rhesus macaques, where these two categories of neurons are referred to as fast spiking units and regular spiking units. Taken together, the results of our study suggest that receptive field structure and response properties of neurons in layer IV of the S1 whisker representation are conserved in marsupials and placental mammals. Further research is necessary to elucidate potential differences in the underlying circuitry, which may be related to species-specific behavior and the lifestyle of the animal.

Title: Perception of partial slips under tangential loading of the fingertip

Authors: *A. BARREA¹, B. DELHAYE², P. LEFEVRE¹, J.-L. THONNARD³;
¹Univ. Catholique De Louvain, Louvain-la-Neuve, Belgium; ²Univ. of Chicago, Chicago, IL

Abstract: When a fingertip is pressed against a surface and moved laterally, slippage does not occur instantaneously but rather in a progressive fashion as established by a classic result in contact mechanics. In previous studies we showed that this transition starts with slips occurring at the periphery of the contact area and then propagating towards its center until the whole contact area slips. The stick ratio (SR) allows us to quantify this phenomenon. It is defined as the ratio of the stuck area over the whole contact area. When slip occurs, SR varies from 1 (no slip) to 0 (full slip). While the mechanical behavior of a slipping fingertip has already been characterized, the perception of this phenomenon by human subjects has not been investigated yet. Here, we designed a psychophysical experiment to examine human ability to detect partial slips. We used a robotic platform to make a transparent glass plate slide tangentially in the radial-ulnar direction against the right index fingertip of five subjects. For each trial, the glass plate was first loaded against the finger pad at a given normal force (NF), which was kept constant during the whole trial. Once NF stabilized at the prescribed level, the plate moved tangentially over a given distance with a constant speed of 5 mm/s. The plate was then moved away from the fingertip. Seven levels of plate displacement were used, ranging linearly from 0.5 mm to 5 mm. Each displacement was repeated eight times for each normal force level (2N or 5N), for a total of 112 trials per subject. At the end of each trial, subjects reported whether or not they detected that the plate had slipped under their fingertip. Position and forces exerted on the plate were recorded during the trials. In addition, images of the finger pad contact were also recorded during each trial through the glass plate using an imaging system embedded in the robotic platform. SR was computed from the recorded images using image processing techniques. Results showed that all subjects reported slippage before full slip, i.e. for an average SR of 0.6 (SD = 0.1), corresponding to a plate displacement of 2.3 mm (SD = 0.6 mm). We also tested three predictors they might have used to detect these slips: the plate displacement, the
tangential force or SR. Among the three predictors, SR appeared as the best one allowing subjects to detect partial slips since it was the only one for which NF had no significant effect on the detection threshold of the subjects. In conclusion, this demonstrates that human subjects are able to detect partial slips at the fingertip under tangential loading.

Disclosures:  A. Barrea: None.  B. Delhaye: None.  P. Lefevre: None.  J. Thonnard: None.

Poster

516. Somatosensory Response Properties

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Topic: D.09. Tactile/Somatosensory Systems

Support: KAKENHI Grant 18020030

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JST-PRESTO

Title: Differential modulation of sensory input from three forearm afferent nerves to the spinal cord of the primate during delayed wrist movements

Authors: *J. CONFAIS1, G. KIM1,2, S. TOMATSU1, T. TAKEI1,2, K. SEKI1,2,3,  
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Abstract: During voluntary movements, descending motor command and sensory reafferent input converge at the level of the spinal circuits, and their interaction determine the net drive to the motoneuronal pool. The sensory afferent signal must thus be regulated, so that it does not interfere with the motor command. Previous reports already showed that the input from pure cutaneous afferent is suppressed during the dynamic epoch of a wrist movement task (Seki et al. 2003). This task-dependent suppression of afferent inputs could be induced by either a general inhibition of afferent inputs irrespective of their modality or receptive area, or by a targeted modulation of specific afferents. According to this latter view, input from distinct modalities or nerves could be differently modulated according to behavioral needs. To examine this possibility, we stimulated (1 to 3 Hz) multiple sensory afferents with different receptive area/
modality in the forearm using implanted cuff electrodes, as macaque monkeys (n=4) were performing a delayed wrist flexion-extension task. The effect of nerve stimulation on spinal interneurons was then computed (peak area of the PSTH) and compared according to task epochs. Neurons in which the peak response started 1.5ms or less after the peak of cord-dorsum volley were identified as a putative first-order interneurons, and were used for further analysis (N=203). The stimulated nerve were the Superficial Radial nerve (SR, N=56), a cutaneous nerve innervating the dorsal aspect of the hand, the Deep Radial nerve (DR, N=95), a proprioceptive nerve innervating the extensor muscles of the wrist and fingers, and the Median nerve (M, N=52), a mixed nerve innervating the palmar aspect of the hand and fingers. The effect of nerve stimulation on first-order interneurons was strongly modulated according to task epoch, and differed according to the stimulated nerve. As in our previous report, the input from the cutaneous nerve SR is suppressed during movement, especially during flexion movements. The input from the mixed nerve M is similarly suppressed during movement. By contrast, the input from the proprioceptive nerve DR is facilitated during movement as well as during the instructed delay preceding it, exclusively during extension movements (that is, the direction corresponding to the activation of the target muscles). Sensory inputs seem thus to be differently modulated according to movement direction and input modality, hinting at a fine-tuned input control mechanism at the spinal level rather than a general suppression. As some of these modulations take place before the onset of the movement, they might be the result of a descending anticipatory command.

Disclosures: J. Confais: A. Employment/Salary (full or part-time); NCNP. G. Kim: None. S. Tomatsu: A. Employment/Salary (full or part-time); NCNP. T. Takei: None. K. Seki: A. Employment/Salary (full or part-time); NCNP.

Poster

516. Somatosensory Response Properties

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 516.09/R13

Topic: D.09. Tactile/Somatosensory Systems

Support: DARPA (N66001-10-C-2010)

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UCSF Wheeler Center for the Neurobiology of Addiction
Title: Predictions for parietal cortex from a neural-network model of state estimation

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Abstract: How does the brain learn to track the dynamical state (position, velocity, etc.) of objects, including one's own limbs, as well as external stimuli? In higher mammals, these skills are clearly adaptive and likely learned, rather than inborn. A central locus of this learning is likely to be parietal cortex. We have recently proposed a computational model for how parietal cortex could learn to perform such state estimation. The model is an artificial neural network that is trained by quasi-Hebbian, unsupervised rules—which appear to be ubiquitous in cortex—to become a good "generative" model of its inputs. The key insight is to include among these inputs the recent activity of the model's own hidden layer, which allows the network to learn temporal dependencies in the data. When the rest of the inputs are the responses of sensory units to the moving stimulus, this means learning, implicitly, the dynamics of the object being tracked. This in turn facilitates optimal inference about its dynamical state. When the input also includes units that report a "copy" of the efferent command, the network learns (again without supervision) to interpret the signal as such, as well as how the command influences the trajectory of the moving object. The resulting inference to the position of that object--in this case, the limb--is again nearly optimal. The solution to the state-estimation problem learned by the network is not obvious; it therefore makes predictions for real neural networks in cortex. Here we examine the nature of that solution; the receptive fields (RFs) learned by the model; and how these compare to electrophysiology in MSTd and in Area 5 of posterior parietal cortex. Learning to track external stimuli from position-encoding units causes the network's hidden units to encode position at various temporal lags--i.e., to be tuned for past positions--rather than, for example, simply encoding the current posterior mean and variance, as in the standard engineering solution, the Kalman filter. The resulting RFs resemble those in MSTd in macaques. Moreover, when the task is instead to track the arm during center-out reaches, and the inputs are both position and efference-copy units, the distribution of the hidden units' temporal lags becomes instead roughly symmetric about zero. A very similar distribution has been found in macaque Area 5, which is thought to be responsible for learning the model of arm dynamics. In fine, the network model and the solutions it learns offer explanations for otherwise puzzling electrophysiology in parietal and related areas.


Poster

516. Somatosensory Response Properties

Location: Hall A
Title: Stick-slip movements of whisker depend on multiple variables - some determined by the touched object and some by the actively touching subject

Authors: *M. OLADAZIMI1,2,3, C. SCHWARZ1,2;  
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Abstract: Friction is an important determinant of touch related perception. In whisker mediated touch, friction leads to stick-slip movements (‘slips’) already with light touch - well in the range of variables that is deemed to govern realistic touches performed by rodents. If slips should ever tell the subject something about the touched surface, the dependencies of occurrence should be uniquely related to the touched object - i.e. any other dependencies, e.g. on the way the touch is performed by the subject, must be either minimized or predicted by the subject. We used natural rat vibrissae mounted on a rotating axis and swept by a stepper motor across surfaces to videotape whisker vibration in the plane of whisking (2 kHz frame rate). Slip events were extracted using threshold speed criteria. The occurrence and kinematic profile of slips dependent on, texture roughness (particle size of sandpaper), whisker identity (C2, C4, C6, E2, A2, γ), touch distance (5mm, quarter and half whisker length from the whisker tip), and sweeping velocity (200-1800 deg/s) was assessed (200 sweeps for each condition). We found dependencies of slip occurrence and kinematics on surface roughness as reported before. Small numbers of events with high slip amplitude/velocity were generated by rough surfaces, while ubiquitous small slips were observed with smooth textures. Importantly though, we also found that slips can be dependent on touch distance and sweeping velocity: greater distance and velocity generated larger slips. From our findings, we predict that two selected textures may evoke the same slip profile if touch dependent variables are set accordingly. Thus, assuming that slip kinematics are decisive for tactile perception, rodents need to carefully adjust their active touch strategy to find out the difference between any two surfaces.

Disclosures: M. Oladazimi: None. C. Schwarz: None.

Poster

516. Somatosensory Response Properties

Location: Hall A
Title: Modulation of somatosensory evoked potentials after transcranial static magnetic field stimulation over primary and supplementary motor cortices

Authors: *H. KIRIMOTO, H. TAMAKI, H. ONISHI; Niigata Univ. of Hlth. & Welfare, Niigata, Japan

Abstract: Introduction: Current research by two groups has reported that the primary motor cortex (M1) in the human brain can be modulated by the application of static magnetic fields, and not only time-varying (electromagnetic) fields, through the scalp (Oliviero et al., 2011; Silbert et al., 2013). Moreover, we previously reported that transcranial static magnetic field stimulation (tSMS) over the sensorimotor cortex (C3) reduces the amplitudes of the N20 component of somatosensory evoked potentials (SEPs) (Kirimoto et al., 2014). In transcranial magnetic stimulation (TMS) and transcranial direct current stimulation (tDCS) studies, the M1 is considered an important target with proven efficacy in chronic pain treatment. On the other hands, supplementary motor area (SMA) is thought to one of the generator of SEPs, however the effect of tSMS on the excitability of SMA has never been examined.

Objective: This study aimed to investigate the possibility of non-invasive modulation of SEPs by the application of tSMS over the M1 and SMA in healthy humans.

Methods: tSMS and sham stimulation over the M1 or SMA were applied to 14 subjects for periods of 15 min. SEPs following right median nerve stimulation were recorded before, immediately after, 5 min after, and 10 min after tSMS from sites C3’ and F3. Using a Gauss meter, magnetic field strength of a cylindrical neodymium magnet (NdFeB) was measured, both through the human skull specimen and directly.

Results: Amplitudes of the P25 and N33 component of SEPs at C3’ significantly decreased immediately after the 15-min period of tSMS over the M1 by up to 20%, returning to baseline by 10 min after intervention. However, tSMS over the SMA did not influence any of the components of SEP amplitude. At a distance of 2-3 cm (rough depth of the cortex), magnetic field strength was in a range between 110-190mT, regardless of the presence or absence of the skull.

Conclusions: Our results suggest that different components of SEPs are reduced according to the tSMS stimulation site: for instance, tSMS over the C3 modulates the N20 component of SEPs, while the amplitude of N33 is affected by tSMS over the M1. This result, showing that tSMS over the M1 can reduce the N33 component of SEPs at C3’ is consistent with repetitive transcranial magnetic stimulation and transcranial direct current stimulation over M1 studies (Lefaucheur et al., 2008; Sugawara et al., 2014). Therefore, tSMS could be a useful tool for modulating cortical somatosensory...
processing. SMA is likely to be a more difficult area to target with tSMS than M1, since it is located in the interhemispheric fissure, which would result in the magnetic field strength of tSMS being attenuated to on-effective levels.

Disclosures: H. Kirimoto: None. H. Tamaki: None. H. Onishi: None.

Poster
517. Axonal Regeneration

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 517.01/R16


Title: Regression of the expression of growth associated genes after chronic nerve injuries

Authors: *T. GORDON1, W. TETZLAFF2;
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Abstract: The regenerative capacity of injured neurons and the regenerative support provided by Schwann cells declines after delayed surgical repair and over time and distance. Injured neurons undergo morphological changes that reflect altered gene expression including the upregulation of regeneration associated genes (RAGs) that include the cytoskeletal proteins, actin and tubulin, and neurotrophic factors such as glial and brain derived neurotrophic factors. The question that we asked is whether this expression is sustained or whether there is a regression with time that might, in turn, account for the progressive reduction in the capacity of chronically injured neurons to regenerate their axons. In Sprague Dawley rats, sciatic nerves were transected and the nerve stumps ligated and sutured to innervated muscle to prevent regeneration for periods of up to 6 months and, in a subgroup of rats, the proximal nerve stump was refreshed and again ligated to prevent nerve regeneration. At 7 days and months after axotomy, the expression of actin, tubulin, neurofilament, and GAP-43 in the motoneurons that supplied the experimental axotomized sciatic nerve and those that supplied the contralateral intact sciatic nerve was determined using in situ hybridization. When expressed relative to the RAG expression in the motoneurons supplying the contralateral intact sciatic nerve, the expression of tubulin, actin and GAP-43 mRNA was increased to a maximum and that of neurofilament was reduced within 7 days. Thereafter the RAG expression of each of the upregulated genes declined and the down-regulated genes increased with an exponential time course to reach basal levels equal to the contralateral sciatic motoneurons. This decline was reversed when the ligated nerves were
refreshed by a transection injury but the expression of the tubulin, actin, and GAP-43 genes declined and the neurofilament genes increased, the rate of the change in expression being significantly faster than after the first injury. These findings demonstrate the transient nature of expression of RAGs and that, whether or not the ligated nerves were refreshed, the exponential decline in the expression reaches basal levels, the decline corresponding with the time course of decline in the regenerative capacity of chronically injured neurons. Therefore, the transient expression of RAGs accounts, at least in part for the relatively short window of opportunity when the regenerative response of injured neurons is maximum and when, in turn, functional return is optimal.


Poster

517. Axonal Regeneration

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 517.02/R17


Title: Chronic electrical muscle stimulation (EMS) immediately after nerve transection and repair increases reinnervated muscle force but does not prevent misdirection of regenerating nerves

Authors: *M. WILLAND, J. CATAPANO, T. GORDON, G. H. BORSCHEL;
Div. of Plastic Reconstructive Surgery, The Hosp. For Sick Children, Toronto, ON, Canada

Abstract: Introduction: Functional recovery after surgical repair of transected nerves is reduced when axons are misdirected to reinnervate muscles other than their original and appropriate targets. Canine models of laryngeal nerve transection injury have suggested that EMS during the period of nerve regeneration promotes reinnervation of original targets. However, these conclusions were based on functional measurements and there remains no direct evidence that the muscles are reinnervated by motoneurons from the original motoneuron pool following EMS. In this study, we investigated whether EMS using a natural activation pattern increases the number of motoneurons regenerating to innervate their original target muscle. Methods: Prior to injury, soleus muscles in two groups of Sprague Dawley rats were injected with True Blue tracer to label the original motoneuron pool in the spinal cord. One week later, the lateral gastrocnemius soleus (LGS) nerve was transected and immediately repaired and the soleus muscle was implanted with intramuscular wire electrodes attached to implanted mini-stimulators. In one group of rats the denervated soleus muscle was subjected to EMS with a paradigm
mimicking the natural activation of the soleus muscle: 12 hours (h) of continuous daily EMS at 20 Hz (10 seconds on, 20 sec off) followed by 12 h of intermittent EMS (10 sec on, 1 h off). Two months after the LGS nerve transection and repair, the motoneurons that had regenerated axons into the soleus nerve were retrogradely labelled with FluoroRuby just proximal to the soleus muscle. The motoneurons of the contralateral uninjured lateral gastrocnemius (LG) and soleus muscle were labelled to compare the spatial distribution of the contralateral motoneuron pool with that of the injured side. A second group of rats, undergoing the same procedure, were used for isometric force recordings. Results: Intramuscular injection of soleus muscle resulted in a labeling efficiency of 75% of the uninjured contralateral side which was labeled using the gold standard well technique. Double labeled motoneurons, representing reinnervated axons that originally innervated the soleus muscle, were no different between groups (EMS: 10.9 ± 2.3 % vs Sham: 15.5 ± 9.9 %). Visualizing 3D spatial distributions of labeled neurons indicated that more axons originated from the LG motoneuron pool which has 3x more motoneurons than the soleus. Muscle twitch forces were greater in the EMS group (33 ± 4.6 % vs 20 ± 2.5%).

Conclusions: In our model, EMS does not counteract the misdirection of nerve regeneration but it does enhance muscle force in line with our previous finding that EMS accelerates muscle reinnervation.


Poster

517. Axonal Regeneration

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 517.03/R18


Support: NS057190

NS087915

Title: Optical activation of cut axons in mouse peripheral nerves enhances regeneration and muscle reinnervation

Authors: *A. W. ENGLISH, P. J. WARD, S. PARK;
Dept Cell Biol., Emory Univ. Sch. Med., Atlanta, GA

Abstract: To evaluate whether increasing neuronal activity is sufficient to promote axon regeneration and functional recovery following peripheral nerve injury, we activated peripheral
axons using optogenetics. In thy-1-ChR2/YFP mice, in which channelrhodopsin is expressed at para-nodai regions of nearly half of the sensory and motor axons in peripheral nerves, neural activation was achieved by application of short (1 ms) pulses of 473 nm light at 20 Hz for one hour prior to transection and surgical repair of the right sciatic nerve. Mice with similar injuries that were untreated (UT) served as controls. All mice were implanted with stimulating cuffs around the right sciatic nerve, distal to the injury site, and EMG recording electrodes in the gastrocnemius and tibialis anterior muscles bilaterally. The time course of axon regeneration and muscle fiber reinnervation were studied from EMG potentials evoked by electrical stimulation of the right sciatic nerve. Recordings were made weekly in awake animals for as long at 18 weeks after nerve injury. Even though treatment with optical stimulation (OS) results in activation of only half of the sensory and motor axons in the sciatic nerve, OS at the time of injury resulted in an earlier reappearance of compound muscle action potentials (M responses) and monosynaptic H reflexes in reinnervated muscles than in UT mice, and a significantly more rapid restoration of the pre-transection M response amplitude. These outcomes are similar to data from animals exposed to one hour of 20 Hz supramaximal electrical stimulation at the time of nerve transection and repair, a treatment which is expected to activate all axons in the nerve. The isolated activation of neurons whose axons have been transected is sufficient to promote enhanced axon regeneration and improved functional recovery after peripheral nerve injury.

Disclosures:  A.W. English: None. P.J. Ward: None. S. Park: None.

Poster

517. Axonal Regeneration

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 517.04/R19


Support: NS057190

Title: Pharmacogenetic enhancement of functional recovery from sciatic nerve transection

Authors: *P. B. JAISWAL, A. W. ENGLISH;
Emory Univ., Atlanta, GA

Abstract: Designer receptors exclusively activated by designer drugs (DREADDs) are a pharmacogenetic tool that could be used to modulate neuronal excitability in the peripheral nervous system. We hypothesized that activation of excitatory (Gq) DREADD by the physiologically inert ligand, clozapine-N-oxide (CNO), will increase the excitability of
DREADD-infected neurons whose axons have been transected and that this will lead to enhanced axon regeneration and improved functional recovery. The gastrocnemius (GAST) muscle of young female Lewis rats was injected unilaterally with AAV9::hM3Dq-hsyn-mCherry (7.6x109 viral genomes/µl). The contralateral muscle served as a control. Six weeks after intra-muscular injection, direct muscle (M) responses and monosynaptic H reflexes were elicited from sciatic nerve stimulation, under isoflurane anesthesia (1-2% in oxygen) before (Baseline) and after intra-peritoneal injection with 1mg/kg CNO (Post CNO). The sciatic nerve was then transected and repaired bilaterally and rats were treated with 1mg/kg CNO by daily intra-peritoneal injection for three days after injury. Electrophysiological data were collected at 2 weeks and 4 weeks after injury. A prolongation of both the latency and duration of evoked responses was observed at 2 and 4 weeks after transection. However, these responses were shorter in the DREADD-infected than in the contralateral GAST muscles. Recovery of elicited responses was observed at 2 weeks after transection injury in animals treated with CNO on the DREADD-infected but not the contralateral GAST muscle. The Baseline amplitude of the maximal M-response was greater on the DREADD infected but not the contralateral GAST at 4 weeks after injury in all animals. Post CNO amplitude of the evoked M-response and H reflex in the DREADD-infected muscle was greater than the contralateral GAST muscle, compared to their respective Baseline, at Intact and 4 weeks after transection. This novel use of DREADD technology in the peripheral nervous system caused sustained increases of neuronal excitability, which was sufficient to produce enhanced recovery of the evoked muscle responses after sciatic nerve transection.

Disclosures: P.B. Jaiswal: None. A.W. English: None.

Poster

517. Axonal Regeneration

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 517.05/R20


Title: A glial cell line derived neurotrophic factor delivery system enhances nerve regeneration in acellular nerve allografts

Authors: *K. TAJDARAN¹, M. D. WOOD¹, M. S. SHOICHET⁴, T. GORDON⁵, G. H. BORSCHEL²;
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Abstract: Purpose: Recently, surgeons have been using decellularized nerve allografts to bridge nerve gaps, but these grafts lack therapeutic levels of neurotrophic factors and therefore do not support regeneration to the same extent as autografts. Here we investigated a local delivery system for glial cell line derived neurotrophic factor (GDNF) controlled release to the implanted decellularized nerve allografts using drug-loaded poly(lactic-co-glycolic acid) (PLGA) microspheres (MS) embedded in a fibrin gel. The hydrogel composite served to localize MS around the nerve allografts and allows sustained GDNF release. Methods: In a nerve gap model, a 10 mm decellularized nerve graft was used to bridge a 5 mm common peritoneal (CP) nerve gap. In negative control groups, animals received no treatment or received fibrin gels with empty MS at both suture sites of the nerve graft. Fibrin gels loaded with MS encasing GDNF with 2 weeks or 4 weeks release period served as the primary experimental groups. Rats receiving nerve isografts served as the positive control group. 8 weeks after repair, nerve regeneration was assessed using retrograde labeling and collecting nerve samples 10 mm distal to the graft for histomorphometric analysis. Results: Regeneration for both motor and sensory neurons in all the groups with GDNF MS treatment and isograft treatment were indistinguishable and significantly higher compared to the negative control groups. Qualitative and quantitative analysis of nerve samples obtained at 10 mm distal from the nerve grafts revealed that groups receiving GDNF MS had similar nerve morphology to the isograft receiving group, with significantly higher myelinated axons present in the nerve cross sections compared with the negative control groups. Fiber frequency analysis indicated enhanced nerve maturity for the GDNF MS and isograft treated groups as the number of fibers with larger diameter (4 µm - 6 µm) was higher compared to the negative control groups with more smaller diameter fibers (2 µm - 4 µm). Conclusion: GDNF local administration using a biodegradable and biocompatible drug delivery system makes the decellularized nerve allografts as effective as the isografts in supporting nerve regeneration. The biomaterial developed in this study has the potential to provide an “off the shelf” alternative in the current management of severe nerve injuries.

Title: Large-diameter sensory neurons require androgen receptor signaling for activity-enhanced axon regeneration

Authors: *P. J. WARD, A. ENGLISH;
Dept of Cell Biology, Emory Univ., Atlanta, GA

Abstract: Signaling through androgen receptors is required for the effects of increased neuronal activity on enhancing axon regeneration following peripheral nerve injury. The targets of androgenic activity required to promote axon regeneration are unknown. Here, we evaluated whether androgen receptor signaling in sensory neurons is necessary for enhancing axon regeneration following nerve transection and repair that is produced by increased neuronal activity. In wild type (WT) mice that received electrical stimulation for 1 hour (20 Hz) prior to sciatric nerve transection and repair, we observed an increased number of retrogradely-labeled, large diameter sensory neurons in the L4 dorsal root ganglia two weeks later, compared to untreated WT mice. We compared these results to the effects of increased activity in Adv-Cre::ARff mice, in which the androgen receptor is knocked out selectively (via tamoxifen administration at 8 weeks of age) in a subset of sensory neurons (primarily large diameter sensory neurons). Although the mice received one hour of 20 Hz electrical stimulation just prior to injury, we observed fewer retrogradely-labeled, large diameter sensory neurons two weeks later. These results suggest that neuronal androgen receptor signaling is required for the effects of activity-induced axon elongation of large diameter sensory neurons following nerve injury.

Disclosures: P.J. Ward: None. A. English: None.
Title: Modulation and inhibition of M-response and H-reflex activity using kilohertz electrical stimulation

Authors: *Y. Patel*, R. J. Butera, A. W. English;
\(^1\)Georgia Inst. of Technol., Atlanta, GA; \(^2\)Cell Biol., Emory Univ., Atlanta, GA

Abstract: Kilohertz electrical stimulation (KES, 3-70 kHz) has been shown to be effective at blocking action potential propagation in whole nerves, which contain both myelinated (A) and unmyelinated (C) fibers. The threshold for C-fiber KES block is lower than the threshold for A-fiber KES block at sufficiently high frequencies. To evaluate whether KES block could be selective towards afferent and efferent fibers in peripheral nerves, we stimulated the sciatic nerve (test stimulation) in urethane-anesthetized mice and measured evoked EMG potentials in the gastrocnemius and plantar foot muscles in the presence or absence of KES. If KES is applied distal to the test stimulation, the direct muscle (M) responses are blocked completely at frequencies from 4-20 KHz. At higher frequencies, KES is ineffective in blocking motor action potential propagation. If KES is applied proximal to the test stimulus, both the M response and the monosynaptic H reflex are blocked at frequencies that are effective in blocking efferent action potentials, but at higher frequencies (20-40 KHz) the H reflex is lost, but the M response persists. These findings are interpreted to mean that KES can be used to produce selective block of action potential propagation in afferent or efferent axons in peripheral nerves. It could prove useful either as an experimental tool or as an experimental treatment to regulate activity in peripheral nerves.

Disclosures: Y. Patel: None. R.J. Butera: None. A.W. English: None.

Poster 517. Axonal Regeneration

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 517.08/S3


Title: Network medicine for retrograde motoneurodegeneration after peripheral nerve lesion

Authors: *D. Romeo-Guitart*, M. Herrando-Grabulosa, T. Leiva-Rodriguez, R. Valls, J. Mas, M. Coma, J. Forés, C. Casas
Abstract: Spinal root traction or avulsion leads to a progressive loss of axotomized motoneurons (MNs) which sometimes avert warranty for surgical nerve repair. Since the molecular mechanisms involved in this neurodegenerative process are non-apoptotic and unknown, we performed an unbiased and comparative proteomic study coupled to System Biology approach for data using two pre-clinical rat models: a Root Avulsion (RA) model that produces retrograde motoneurodegeneration, and a Distal Axotomy (DA) model that promotes MNs survival and regeneration. We generated topological maps with associated mathematical algorithms for protein interactions that characterized each condition. We wanted to find patentable drug combinations capable to change the degeneration-related map into the regeneration-based one. For that purpose, we used a computational tool called the therapeutic performance mapping system (TPMS) technology which is based on artificial neural intelligence to predict biological responses to different stimuli. We selected drug combinations with the highest scores and validated its neuroprotective potential. The treatment also led to a reduction of gliosis in the ipsilateral side of the spinal cord and promoted the apparition of regenerative markers such as GAP43. In addition, the use of TPMS allowed the prediction of the mechanisms of action (MoA) for each combination. Thus, we validated that the treatment with combination number 3 was capable to regulate key proteins related to MN survival. In conclusion, we verified that the present overall approach is promising as novel methodology to design effective treatments for complex pathological conditions.


Poster
517. Axonal Regeneration

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 517.09/S4


Support: MINECO BFU2012-33975

Junta de Andalucía P10-CVI-6053
Title: VEGF administration maintains normal discharge characteristics on axotomized abducens motoneurons in adult cats

Authors: *R. M. DE LA CRUZ, P. M. CALVO, A. M. PASTOR;
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Abstract: VEGF was discovered by its effects on the vasculature, such as vasculogenesis, angiogenesis and increased capillary permeability. However, recent evidences point to this molecule as an important neurotrophic factor, particularly on motoneurons. For example, transgenic mice with reduced levels of VEGF develop an adult-onset motoneuronal degeneration similar to amyotrophic lateral sclerosis. Moreover, the exogenous administration of VEGF to animal models of this disease leads to beneficial effects. However, information linking this factor to physiologival or synaptic activity on motoneurons in vivo is lacking. Our objective was to evaluate the action of VEGF exogenous administration on lesioned motoneurons in order to determine to what degree firing properties and synaptic inputs could be protected by this factor. First of all, we carried out an immunocytochemical study that determined that all abducens motoneurons are endowed with the two types of receptor of the VEGF: VEGFR1 (or Flt-1) and VEGFR2 (or Flk-1). Secondly, we prepared animals for the chronic recordings of identified abducens motoneurons under alert conditions. In control, these motoneurons show a typical tonic-phasic discharge pattern that correlates with eye position and eye velocity, giving rise to two neuronal sensitivities, eye position sensitivity (k, in spikes/s/degree) and eye velocity sensitivity (r, in spikes/s/degree/s), respectively. Axotomized cells discharged at lower rates both during fixations and saccades, yielding a significant decrease in both parameters. VEGF was administered through a home-made devise that allowed to introduce the distal end of the VI nerve on a camera through which the factor was delivered (0,2 µg/kg on alternated days). Axotomized motoneurons treated with VEGF showed normal firing characteristics during the different types of eye movements and presented normal k and r values. Since the different types of eye movement relies on particular synaptic inputs, the present data are the first to describe that VEGF completely prevents the firing and synaptic alterations on axotomized motoneurons.

Disclosures: R.M. De La Cruz: None. P.M. Calvo: None. A.M. Pastor: None.

Poster

517. Axonal Regeneration

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 517.10/S5

Title: Peripheral injury induces Ca\textsuperscript{2+} permeable AMPA receptor-mediated maladaptive spinal plasticity

Authors: *J. R. HUIE\textsuperscript{1}, K. MORIOKA\textsuperscript{1}, E. STUCK\textsuperscript{1}, D. FONG\textsuperscript{1}, L. VAN CITTERS\textsuperscript{1}, C. GUANDIQUE\textsuperscript{1}, J. HAEFELI\textsuperscript{1}, V. DEGOS\textsuperscript{2}, M. MAZE\textsuperscript{2}, H. SU\textsuperscript{2}, A. FERGUSON\textsuperscript{1};

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Abstract: Nociceptive input from peripheral injury can lead to the development of chronic neuropathic pain, impaired cognitive function, and perturbations in locomotor function. The expression of these lasting changes in the central nervous system suggests that peripheral injury broadly impacts CNS plasticity. A large body of work has demonstrated that peripheral nociceptive input can alter dorsal horn neurons through cellular mechanisms that are similar to those underlying hippocampal learning and memory, inducing a form of ‘pain memory’. A key mediator of these effects on pain plasticity is the increased expression of calcium-permeable, GluA2-lacking AMPA receptors (CP-AMPARs). It is unknown whether peripheral injury also induces a similar mechanism in ventral motor neurons. In this study we used a tibia fracture model to determine whether spinal motor neurons are susceptible to CP-AMPAR-mediated maladaptive plasticity induced by peripheral musculoskeletal injury. Wild-type C57BL/6J mice received open tibia fracture of the right hind limb under isoflurane anesthesia. Control animals (n=5) received equivalent anesthesia without tibia fracture. Lumbar spinal cord was then extracted either 30 minutes (n=4) or 6 hours (n=6) following tibia fracture. Systematic algorithmic sampling of lumbar motoneurons was performed on histological sections, followed by unbiased automated image analysis of high-resolution 3D confocal images. Quantification of GluA1 and GluA2 AMPA receptor subunit puncta revealed no significant change in tibia fracture animals at 30 minutes post-injury compared to control animals, but did show a significant increase in both synaptic and extrasynaptic GluA1 expression at 6 hours post-injury. There was no significant increase in GluA2 at 6 hours post-injury, suggesting an increase in CP-AMPAR expression. Interestingly, the increase in GluA1 was observed in motor neurons both ipsilateral and contralateral to the injured hindlimb, indicating a broad effect on central spinal cord plasticity. These findings provide evidence for a synaptic mechanism underlying...
maladaptive spinal plasticity in motor neurons in response to peripheral bone fracture, and may provide a spinal CNS target for promoting recovery of neural function after such injuries.


**Poster**

**517. Axonal Regeneration**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program/#/Poster#: 517.11/S6**

**Topic:** C.04. Neurodegenerative Disorders and Movement Disorders

**Support:** NIH Grant NS40433

**Title:** Investigation of the neuroprotective actions of CD4+ T cells and interleukin-10 after facial nerve axotomy in mice

**Authors:** *D. N. OLMSTEAD*1,2, M. M. HAULCOMB1, N. A. MESNARD-HOAGLIN1,4, R. J. BATKA1, N. D. SCHARTZ1, V. M. SANDERS5, K. J. JONES3; 1Anat. and Cell Biol., Indiana Univ. Sch. of Med., Indianapolis, IN; 2Res. and Develop., Richard L Roudebush VAMC, Indianapolis, IN; 3Neurosci. Program, Loyola Univ. Med. Ctr., Maywood, IL; 4Res. and Develop., Hines VA Hosp., Hines, IL; 5Mol. Virology, Immunology, and Med. Genet., The Ohio State Univ., Columbus, OH

**Abstract:** Facial nerve axotomy (FNA) in immunodeficient mice causes significantly more motoneuron loss than in wild type (WT) mice, indicating that the immune system serves a neuroprotective function. Further studies reveal that CD4+ T cells and interleukin-10 (IL-10) are both critical for motoneuron survival after injury, but the neuroprotective IL-10 does not come from CD4+ T cells. The objective of this study is to investigate both the roles of CD4+ T cells and IL-10 in neuroprotection after FNA. To study the neuroprotective effects of CD4+ T cells, WT, immunodeficient recombinase-activating gene-2 knockout (RAG-2 KO), and CD4+ T cell-reconstituted RAG-2 KO mice received a FNA, and qPCR analysis of the facial motor nucleus was conducted to compare gene expression profiles. Expression of genes associated with axonal regeneration, glial activation, cell death receptor pathways, and inflammation was measured. Across the three experimental groups, there was no significant difference in the axonal regeneration or cell death receptor gene expression profiles after axotomy. However, there was a significant decrease in glial activation in RAG-2 KO mice compared with WT and CD4+ T cell-
reconstituted RAG-2 KO mice. Additionally, tumor necrosis factor-alpha (TNFα) expression was significantly decreased in RAG-2 KO mice compared to WT and reconstituted mice, implying that TNFα may also benefit motoneuron survival. Overall, these data suggest that CD4+ T cells improve motoneuron survival by modulating the glial response to axotomy. Additionally, the expression of TNFα for the first 2 weeks after injury may be critical for attracting and activating the immune response that leads to neuroprotection. Further studies will be done to explore the importance of TNFα in immune-mediated neuroprotection. To determine the cellular source of IL-10, a mouse model that expresses IL-10 protein hybridized to green fluorescent protein (GFP) received a FNA, and fluorescent immunohistochemistry was conducted to determine colocalization of the IL-10/GFP protein with cellular markers for astrocytes and microglia. The results showed that microglia, and not astrocytes, produce IL-10 after FNA. Time-course analysis revealed that IL-10 is expressed constitutively, and production peaks at 10 days post-axotomy. Overall, these data support the hypothesis that CD4+ T cells interact with the glia in the facial motor nucleus to regulate their activation post-axotomy and induce microglia to produce IL-10, generating an anti-inflammatory environment that promotes motoneuron survival.

Abstract: Amyotrophic lateral sclerosis (ALS) is a devastating progressive disease involving the loss of motor neurons (MN), which ultimately results in paralysis and death. The superoxide dismutase 1 (SOD1) transgenic mouse model of ALS exhibits similar clinical progression of pathology, including pre-symptomatic, symptomatic, and end stages. Disease onset is thought to begin during the pre-symptomatic stage when axons of spinal MNs retract from target musculature through axonal die-back. Prior research indicates denervation of hindlimb muscles occurs by 47 days of age in SOD1-G93A mutant mice, with progressive loss over time. This early pathological period may represent both a key mechanistic stage in disease progression and an optimal window of time to assess potential therapies for efficacy with clear anatomical outcome (denervation) as a measure of treatment effects. Our data show similar gastrocnemius muscle innervation between wild-type and 35 day old SOD1-G93A mice, but significant gastrocnemius muscle denervation in 47 day old SOD1 mice compared to wild-type (p < 0.05) and 35 day old SOD1 (p < 0.05) mice based on quantification of neurofilament and synaptophysin, a pre-synaptic marker, co-localization with α-bungarotoxin, which labels post-synaptic acetylcholine receptors (AchRs). We have already performed systemic daily therapy with medium conditioned (CM) in culture with adipose-derived stem cells (ASCs) beginning at symptom onset (110 days of age). Our results showed that daily ASC-CM ameliorated characteristic progressive motor neuron loss in the lumbar spinal cord (p < 0.005), and prolonged survival (p < 0.05) compared to SOD1-G93A mice treated with vehicle medium. Our current pre-symptomatic disease onset data sets the stage for testing whether this systemic ASC-CM therapeutic paradigm can prevent gastrocnemius denervation and promote long-term functional and survival benefits in the SOD1-G93A mouse model of ALS.


Poster
518. Cerebellum: Cortex and Nuclei Neurophysiology
Location: Hall A
Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM
Program#/Poster#: 518.01/S8
Topic: D.14. Cerebellum: Central Physiology
Support: MH 46904
MH 74006
Title: Dealing with uncertainty: binary decision making in the cerebellum
Authors: *A. KHILKEVICH, J. E. CANTON-JOSH, M. D. MAUK;*
Univ. of Texas At Austin, Austin, TX

Abstract: The world is an uncertain place. Brain systems must operate properly, despite noisy and variable sensory inputs. The cerebellum is known to receive vast sensory and proprioceptive inputs, thus it is critical to know how the cerebellum deals with uncertainty in these inputs. The question is the following: if the cerebellum has learned a response of a certain size to a particular input, how should it respond to an input that is partially similar to the trained input? To answer this question, we used a combination of cerebellar-dependent behavior, full control over inputs to the cerebellum and simultaneous in-vivo tetrode recordings from the cerebellar cortex. Specifically, New Zealand albino rabbits were implanted with two stimulation electrodes in the middle cerebellar peduncle and a 16 tetrode microdrive in the left anterior lobe of the cerebellar cortex. They were trained using a delay eyelid conditioning paradigm using electrical stimulation of mossy fiber inputs (100 Hz, 100 µs pulse width, 500 ms burst width, 100 µA) as the conditioned stimulus (CS). Such design gave us full control over the intensity and temporal characteristics of input to the cerebellum. Animals were trained until they showed robust conditioned responses (CRs) of a target amplitude. After that, we introduced CS-alone probe trials, where input to the cerebellum was systematically altered in three different ways from the original CS. We either decreased the frequency of stimulation, shortened the duration of the stimulus or decreased the current on the electrode used for training while adding competing stimulus through previously unused electrode. Our results show that under all conditions the cerebellum makes a binary choice at the behavioral level: either to make a CR with amplitude and timing appropriate to its previous training, or to not make a response at all. In all three cases, as the stimulus on probe trials became more distinct from the original CS, the probability of observing a CR decreased. However, the size of CR was independent from the CR probability, indicating two separate mechanisms for 1) the decision of whether the cerebellum should make a CR and 2) the size of CR on trials when it is present. Simultaneous recordings from Purkinje cells (PCs), the output neurons of the cerebellar cortex, allowed us to localize such decision to be the result of cerebellar cortex computation. Recordings from other cerebellar cortex neurons suggest that the decision about the presence or absence of CR is made prior to PCs, but PCs amplify this difference to a binary choice. Together, these data demonstrate a novel computational principle that the cerebellum implements when faced with uncertainty in its inputs.


Poster

518. Cerebellum: Cortex and Nuclei Neurophysiology

Location: Hall A
Title: Following learning-related activity in Purkinje cells and interpositus nucleus during the transition from delay to trace eyelid conditioning

Authors: *H. E. HALVERSON¹, A. KHILKEVICH², M. D. MAUK³;
¹Ctr. for Learning and Memory, Univ. of Texas At Austin, Austin, TX; ²Ctr. for Learning and Memory, The Univ. of Texas at Austin, Austin, TX

Abstract: The cerebellar cortex and anterior interpositus nucleus are necessary for acquisition and expression of properly timed conditioned eyelid responses (CRs). Much of what is known about the cerebellar mechanisms supporting eyelid conditioning has focused on delay conditioning, although the same areas of cerebellum necessary for delay also appear to be engaged during trace conditioning. It is not known whether Purkinje cells and/or interpositus neurons contribute to CR expression during trace conditioning in a similar way as their contributions during delay conditioning. The rules dictating which neurons are contributing during CR expression could be different for each paradigm with respect to population activity or at the single neuron level. Before training a 12 tetrode hyperdrive array was implanted dorsal to the ipsilateral anterior lobe or the anterior interpositus nucleus. Neuronal activity was recorded during initial CR expression with delay conditioning using a 500 ms tone conditioned stimulus (CS) and 50 ms periorbital shock (2-3 mA) unconditioned stimulus (US). After training with delay conditioning, rabbits were switched to trace conditioning with the same 500 ms tone and 500 ms stimulus free trace interval to obtain recordings from the same population of Purkinje cells and interpositus neurons. A few individual neurons in each area were followed throughout the switch from delay to trace conditioning. Eyelid Purkinje cells were identified by the presence of US-evoked complex spikes. Eyelid interpositus neurons were identified by stimulating through tetrodes showing learning-related activity and evoking eyelid movements. Recordings in each area showed that at the population level the same group of neurons are involved with the expression of properly-timed delay and trace CRs. Following individual neurons during the switch from delay to trace conditioning provided compelling evidence that the same neurons in each area were also involved with generating well-timed CRs for both paradigms. The same neurons are engaged during delay and trace conditioning, however learning-related responses in each area of the cerebellum changed slightly to produce appropriately timed CRs during each paradigm, even at the individual neuron level.

Poster

518. Cerebellum: Cortex and Nuclei Neurophysiology

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 518.03/S10

Topic: D.14. Cerebellum: Central Physiology

Support: IEF Marie Curie Fellowship FP7

Wellcome Trust

Title: Functional characterization of identified mossy fiber synaptic inputs in the input layer of cerebellar cortex

Authors: *F. LANORE¹, A. HANTMAN², A. SILVER¹;
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Abstract: The input layer of the cerebellar cortex receives sensory inputs from almost every sensory system, together with information from different cortical areas about motor commands and sensory feedback. Sensory and motor command signals are conveyed by mossy fibers (MFs), which arise from multiple brainstem nuclei and make glutamatergic synapses onto inhibitory Golgi cells (GoCs) and excitatory granule cells (GCs) in the granule cell layer. GoCs provide the sole neuronal source of inhibition to GCs. Although the gross structure of the cerebellum is well characterized, the fine scale connectivity between MFs arising from the different precerebellar nuclei and GoCs and GCs have only partially been studied. Fine scale connectivity is important as it ultimately determines how sensory and motor signals are combined and transformed. To investigate the connectivity and functional properties of MFs arising from specific precerebellar nuclei we expressed channelrhodopsin-2 (ChR2) in spinal trigeminal nuclei (Sp5; which conveys sensory information about the face) or in the pontine nucleus (PGN; which carries motor and sensory signals from the cortex) using an adeno-associated virus (AAV) under a synapsin promoter. We recorded light-evoked excitatory synaptic responses in voltage-clamped GoCs and GCs in acute slices containing Crus I and II regions. In the absence of GABA and Glycine receptor antagonists, GCs innervated by Sp5 or PGN MF inputs also received a rapid inhibitory synaptic response from GoCs activated by the same MF inputs indicating the presence of input specific feedforward inhibition. The average amplitude of unitary MF-GC EPSCs arising from Sp5 was larger than those from the PGN (Sp5:...
81 ± 18 pA and PGN: 34 ± 6 pA; P<0.05, unpaired t-test). Moreover short-term synaptic depression of MF-GC EPSCs was larger for Sp5 MFs than for the PGN MFs at 20 and 50 Hz. In contrast, MF-GoC EPSCs exhibited little short-term depression irrespective of their origin. Our results have begun to reveal the fine scale connectivity rules and functional properties of identified MF inputs, thereby providing a better understanding of how incoming information is combined and transformed in the cerebellum.

Disclosures: F. Lanore: None. A. Hantman: None. A. Silver: None.

Poster

518. Cerebellum: Cortex and Nuclei Neurophysiology

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#/Poster#: 518.04/S11

Topic: D.14. Cerebellum: Central Physiology

Support: NIH Grant NS084996-01

Title: Multimodal sensorimotor processing in mammalian cerebellar granule cells

Authors: *T. K. DOYKOS, B. D. HOUCK, A. L. PERSON;
Univ. of Colorado Denver, Aurora, CO

Abstract: Theoretical models suggest that cerebellar computations benefit from access to outgoing motor commands, called corollary discharge (CD), to quickly and accurately control coordinated movements. The nucleocortical pathway, which carries collaterals of premotor output neurons to the cerebellar cortex, serves as an experimentally accessible CD pathway, allowing empirical tests of the role of CD in sensory processing. We used both in vitro and in vivo physiological approaches in mice to test the role of nucleocortical CD information in granule layer processing. In acute cerebellar mouse brain slices, optogenetically evoked nucleocortical EPSCs and IPSCs were recorded in adult granule cells. Surprisingly, we found a complete lack of overlap between cells exhibiting evoked monosynaptic EPSCs (7/13) and polysynaptic IPSCs (6/13), arguing against a simple time-windowing role for feedforward inhibition by cerebellar Golgi cells. To test how the nucleocortical pathway influences sensory processing in vivo, we made extracellular, single-unit recordings from granule cells in ketamine/xylazine anesthetized mice while stimulating cutaneous receptors with an air puff (200 ms; ~150 kPa) to the whiskers with and without concurrent optogenetic activation of nucleocortical terminals (~30-70 mW/mm²). We found that granule cell sensory responses were sensitive to coincident optogenetic activation of nucleocortical motor afferents. Individual cells...
showed sublinear (5/32), linear (31/32) and/or supralinear interactions (9/32) depending on the relative timing of motor and sensory stimuli. Our experiments demonstrate that the cerebellar granule layer exhibits diverse multimodal integrative properties.


Poster

518. Cerebellum: Cortex and Nuclei Neurophysiology

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 518.05/S12

Topic: D.14. Cerebellum: Central Physiology

Title: Granular cells CRFR1 depletion expedites cerebellar associative learning

Authors: *G. EZRA-NEVO1,2, H.-J. BOELE3, M. TSOORY1, A. CHEN1,2;
1 Weizmann Inst. of Sci., Rehovot, Israel; 2 Max Planck Inst. of Psychiatry, Munich, Germany; 3 Erasmus Univ., Rotterdam, Netherlands

Abstract: Corticotropin-releasing factor (CRF), and its cognate receptor, CRF receptor type 1 (CRFR1) play an important and well-established role in the response to stressful challenges. The expression of CRF and CRFR1 in the cerebellar system, including in the granular cell (GrC) layer and in mossy fibers innervating it, have been reported in many species. However, the emotional and cognitive role of the cerebellar CRF system remains elusive. In order to explore the behavioral role of CRFR1 expression in GrCs, we have established a new genetic mouse model specifically depleted of CRFR1 in these cells (GrCs-CRFR1KO), which accounts for more than 90 percent of CRFR1 expressed in the cerebellum. GrCs-CRFR1KO did not show altered baseline motor behavior when compared to control littermate. However, when tested in specific cerebellar dependent classical conditioning, namely delay eye-blink conditioning (EBC), GrCs-CRFR1KO mice presented accelerated learning compared to control mice. This phenotype was dissociated from anxiety related behaviors. GrCs-CRFR1 depletion was correlated with changes in several genes expression, including genes related to intra-cellular calcium homeostasis. We conclude that GrCs CRFR1 plays a role in cerebellar-specific learning, and may be linked to intra-cellular calcium dependent mechanisms. Our findings shed light on the interplay between stress related central components and classical conditioning.

518. Cerebellum: Cortex and Nuclei Neurophysiology

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 518.06/S13

Topic: D.14. Cerebellum: Central Physiology

Support: Wellcome Trust WT094077MA

Title: Synaptic interactions between interneurons and Purkinje cells in cerebellar cortex in vivo

Authors: *C. ARLT, C. D. WILMS, M. HAUSSER;

Abstract: Molecular layer interneurons (INs) make monosynaptic GABAergic synapses with postsynaptic Purkinje cells in the molecular layer of the cerebellar cortex. In vitro experiments have shown that interneurons are excited directly by parallel fiber input, and indirectly by glutamate spillover from climbing fibers, and that they in turn can inhibit the spike output of Purkinje cells. How single INs are excited and interact with Purkinje cells under in vivo conditions remains poorly understood. Here we addressed these issues using simultaneous cell-attached recordings from IN-PC pairs guided by two-photon imaging in head-fixed mice under isoflurane anesthesia. We recorded both spontaneous as well as sensory-evoked spiking activity, using an airpuff to the ipsilateral perioral region. Crosscorrelograms of spontaneous IN spikes and PC simple spikes showed a spike-triggered decrease in PC spiking, time-locked to the IN spikes (n=12 out of 56 pairs, mean reduction to 70% of baseline rate). Triggering single IN spikes with direct stimulation of the interneuron resulted in a similar decrease of PC firing (n=6), demonstrating that single INs can efficiently inhibit their targets. Crosscorrelograms of PC complex spikes and IN spikes revealed an increase of IN activity triggered on complex spikes (n = 26 out of 56 pairs, mean increase of 65% above baseline rate), indicative of glutamate-spillover-mediated IN depolarization. Analysis of complex spike waveforms showed that the increase in IN rate scales with complex spike duration and spikelet number. Sensory stimulation could trigger fast excitatory responses in both INs and PCs (n=11 out of 14 pairs) and could synchronize IN-PC pairs. In some cases, PCs responded mainly with inhibition to the sensory stimulus (n=3 out of 11). A trial-by-trial analysis revealed that trials in which the IN responded to the stimulus had a higher baseline IN firing rate, and that sensory-evoked IN spikes could strongly reduce the fast excitatory response in PCs, effectively gating the excitatory PC response to sensory stimulation. These results demonstrate that INs are activated by both parallel fiber and climbing fiber activity in a graded manner and that they exert powerful influence on PC output both during spontaneous activity and during sensory processing. Our findings put the INs
in a position to efficiently modify PC output during climbing fiber-induced plasticity and thereby are consistent with predictions from adaptive filter models of cerebellar function.

Disclosures: C. Arlt: None. C.D. Wilms: None. M. Hausser: None.

Poster

518. Cerebellum: Cortex and Nuclei Neurophysiology

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

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Topic: D.14. Cerebellum: Central Physiology

Support: NIH Grant NS39395

Title: Responses of Purkinje cells in Crus I/II to repetitive whisker pad stimulation in anesthetized mice

Authors: *S. BROWN, I. M. RAMAN;
Neurobio., Northwestern Univ., Evanston, IL

Abstract: The rodent whisker system is extensively represented in the cerebellar cortex in lobules Crus I/II and provides a context for studying sensory information processing by the cerebellum. To study how input to the whisker pad is represented in Purkinje neurons of Crus I/II, we have made in vivo somatic and dendritic whole-cell recordings from Purkinje cells in ketamine/xylazine anesthetized mice and measured responses to air puff stimulation (25 ms, 10 PSI) of the ipsilateral whisker pad. Purkinje cell recordings were confirmed by the presence of both simple and complex spikes. Consistent with previous reports, Purkinje cells in this region show robust responses to passive stimulation of the whisker pad. We found that, in response to a single air puff stimulation, a subpopulation of Purkinje cells produced a well-timed inhibitory postsynaptic potential (IPSP) (~30 ms latency relative to the onset of stimulation, 20-30 ms duration) followed by a barrage of excitatory postsynaptic potentials (EPSPs), which could be resolved in records in which Purkinje cells spontaneously hyperpolarized. As a result of the short latency IPSP and longer-lasting EPSPs, Purkinje cell spiking was reorganized such that a typical response consisted of a pause in simple spiking followed by a well-timed resumption of firing. In cells with lower baseline rates, the post-inhibitory simple spikes tended to include a cluster of action potentials that exceeded the baseline rate; the relative increase post-inhibitory in firing rate was greater in cells with lower baseline rates. To start to investigate how Purkinje cells in Crus I/II transmit more complex patterns of stimuli, we recorded responses to trains of five consecutive air puffs at stimulus frequencies of 1, 2, 4, and 8 Hz. At lower stimulation...
frequencies, the IPSP response characteristics were constant for each stimulus in the train. At higher stimulation frequencies, the IPSP response became smaller in amplitude and less reliable with successive stimuli. The decrement of the IPSP at higher stimulation frequencies resulted in a decrease in the reliability of the pause in simple spiking associated with the stimulus. Thus, over this frequency range, Purkinje cells showed adaptation to this type of somatosensory stimulation. These data provide evidence for a major role of inhibition in setting the strength and pattern of Purkinje cell responses to repetitive whisker pad stimulation.

Disclosures: S. Brown: None. I.M. Raman: None.

Poster
518. Cerebellum: Cortex and Nuclei Neurophysiology

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

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Topic: D.14. Cerebellum: Central Physiology

Support: NIH grant 5R01DC00415413

NIH grant 5R01NS07240605

NSF GRFP grant DGE-114747

Title: Inhibition of Purkinje cell firing induces motor learning

Authors: *H. L. PAYNE¹, B. NGUYEN-VU², J. L. RAYMOND²;
²Neurobio., ¹Stanford Univ., Stanford, CA

Abstract: Recent evidence suggests that the simple spike output from cerebellar Purkinje cells (PCs) can instruct motor learning (Lee et al., 2015; Nguyen-Vu et al., 2013). Here, we analyzed the role of decreases in Purkinje cell firing in the induction of vestibulo-ocular reflex (VOR) learning. We optogenetically controlled PC firing using transgenic mice expressing ArchT under control of the PC-specific L7 promotor. Inhibition of PCs in the cerebellar flocculus with 532 nm light elicited robust eye movements. To assess whether pauses in PC output are sufficient to induce motor learning, L7-Arch mice underwent 30 min of training consisting of a vestibular stimulus (rotation about an earth-vertical axis at 1 Hz, with 10°/s peak velocity) paired with optogenetic inhibition of PCs in the flocculus (500 ms light pulses). Light was delivered either during head turns towards the side of stimulation ("ipsiversive PC suppression"), or away from the side of stimulation ("contraversive PC suppression"). Before and after training, the VOR was tested by measuring the eye movements elicited by the vestibular stimulus in the absence of the
optogenetic stimulus. Compared to a habituation control with only the vestibular stimulus delivered during training, ipsiversive PC suppression induced a significant increase in the gain of the VOR, measured in the absence of optogenetic stimuli. In contrast, contraversive PC suppression induced no change in the VOR relative to control. Taken together with previous findings, this indicates that activation or suppression of floccular PCs are both sufficient to induce learned increases in VOR gain, and that such learning depends on the specific timing of PC modulation relative to the sensory input. We assessed the site of plasticity underlying PC-induced learning. Before and after training, Purkinje cells in the flocculus of L7-Arch mice were bilaterally inactivated for 60 s while a vestibular stimulus was delivered to test the flocculus-independent component of the VOR. Preliminary results indicate that the learning induced by ipsiversive PC suppression is mediated, at least in part, by plasticity in the flocculus-independent VOR pathway. In contrast, the flocculus-independent component of the VOR was relatively unchanged after training with contraversive PC suppression. Therefore, pauses in PC simple spikes can induce learning via plasticity downstream of the cerebellar cortex.

Disclosures: H.L. Payne: None. B. Nguyen-Vu: None. J.L. Raymond: None.

Poster

518. Cerebellum: Cortex and Nuclei Neurophysiology

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 518.09/S16

Topic: D.14. Cerebellum: Central Physiology

Support: HHMI

Title: Skilled voluntary motions activate a cerebellar state of intrinsically synchronized neural dynamics

Authors: *M. J. WAGNER¹, J. SAVALL¹, J. Li¹, M. J. SCHNITZER¹⁲; ¹Stanford Univ., Stanford, CA; ²HHMI, Stanford, CA

Abstract: It has long been hypothesized that to coordinate voluntary movements the cerebellum enters a physiologic state of internally synchronized neural dynamics. This predicts cooperative neural activity anticipating movement initiation, greater neural synchronization promoting superior motor performance, and encoding of motor information by concerted neural activity that cannot be fully deciphered using single cell recordings. We tested these predictions by two-photon calcium imaging of cerebellar Purkinje neurons’ complex spiking patterns in mice making targeted forelimb reaches on a robotic manipulandum. Our custom-designed robot can
record and manipulate arm movements in real-time. In the basic task version, mice moved the robotic arm to a target region to receive a water reward. In more advanced versions, to permit studies of unexpected self-motion we used real-time control of the robot to physically perturb the arm-reaching trajectory. Mice made hundreds of reaches per behavioral session. From the two-photon imaging data we determined the timing of individual complex spikes to an accuracy of 6-11 ms (s.d.); this accuracy was essential for evaluating whether the cells were using concerted codes. Reaching evoked large-scale synchrony near movement initiation followed by coordinated neural silence peaking after initiation. By using shuffled datasets, our analyses showed that task- or sensory-driven modulations of complex spiking do not account for the synchrony increases. This insufficiency points to internal synchronization as being necessary to account for the concerted dynamics. Testifying to the importance of internal synchrony for movement quality, the greater the neural synchrony at reaching onset, the straighter and less variable was the ensuring arm motion. When the robot unexpectedly perturbed reaching mid-trajectory, concerted spiking conveyed information about the perturbation direction that was absent from single cell spiking patterns. Thus, the cerebellum coordinates skilled motions in a large-scale, internally synchronized state. Other motor circuits might also employ intrinsic synchrony and silence to choreograph movement.

Disclosures: M.J. Wagner: None. J. Savall: None. J. Li: None. M.J. Schnitzer: None.

Poster

518. Cerebellum: Cortex and Nuclei Neurophysiology

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Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 518.10/S17

Topic: D.14. Cerebellum: Central Physiology

Support: R01NS078311

R01EY019258

R01EY019258

F31NS090860

Title: Encoding of action by the Purkinje cells of the cerebellum

Authors: *D. J. HERZFELD¹, Y. KOJIMA², R. SOETEDJO², R. SHADMEHR¹;
Abstract: Execution of accurate eye movements depends critically on the cerebellum, as evidenced by inactivation and lesion studies, suggesting that Purkinje cells (P-cells), may predict the state of the eye. Yet, this encoding has remained a long-standing puzzle: firing of P-cells show little consistent modulation with respect to saccade speed or direction, and critically, lasts significantly longer than duration of a saccade. Given that the cerebellum is thought to play a critical role in terminating saccades, how can P-cells be involved in predicting or controlling the eye if their response persists so much longer than the saccade? We analyzed simple spike activity of 72 oculomotor vermis (OMV, cerebellar lobules VI and VII) P-cells from five monkeys during saccadic eye movements. Our population included cells that during the saccade period exhibited either increased activity (bursting; n=39), or decreased activity (pausing; n=33). These cells project to the caudal fastigial nucleus (cFN), where about 50 P-cells converge onto a single cFN neuron. We computed the population response by choosing 50 P-cells randomly (both bursting and pausing cells) and then computed the change in firing rate of the population. When we averaged across all saccade directions, the time course of the population activity predicted the time course of saccade speed in real-time; an encoding that was not present in either population alone. A population response makes the fundamental assumption that a random selection of the recorded neurons converges onto a single output neuron. However, in the cerebellum the P-cells that project onto a single cFN neuron are not selected randomly, but are likely organized by their inputs from the inferior olive. In this scenario, the olive projections divide the P-cells into modules where projects onto one cFN neuron. We found that if we organized the simple spikes of the P-cells based on each P-cell’s complex spike (CS) properties, additional features of the population response were revealed. When we aligned the simple spike activity of each P-cell to a coordinate system that depended on that cell’s CS tuning, the result unmasked a pattern of inhibition at cFN that encoded saccade speed and direction via a multiplicative gain-field. Therefore, our results suggested three new ideas: reliable encoding of saccade metrics does not occur in the firing of individual P-cells, but via synchronized inputs of bursting and pausing cells onto cFN; in this encoding, speed and direction are multiplicatively represented via a gain-field; and the anatomical projections of P-cells to cFN neurons are not random, but organized by the CS tuning of the P-cells.

Disclosures: D.J. Herzfeld: None. Y. Kojima: None. R. Soetedjo: None. R. Shadmehr: None.

Poster

518. Cerebellum: Cortex and Nuclei Neurophysiology

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 518.11/S18
Title: Application of antagonist drugs of inhibitory receptors in the macaque ventral paraflocculus changes the response of Purkinje cells during oculomotor behaviors

Authors: *P. M. BLAZQUEZ, T. A. YAKUSHEVA;
Otolaryngology, Washington Univ., Saint Louis, MO

Abstract: Inhibition is ubiquitous in the cerebellar cortex. In the granular layer, Golgi cells release GABA and glycine to inhibit information transfer from mossy fibers (mf) to granule cells at the glomerulus. In the molecular layer, GABA released by stellate and basket cells act over Purkinje cells (PCs), which are themselves GABAergic interneurons that contact nearby PCs. Our current understanding of the role of inhibition in cerebellar cortex function has come mainly from studies carried out in in-vitro and anesthetized preparations. These studies have greatly advanced our understanding of cerebellar signal processing, but they face the challenging task of extrapolating their result to the awake animal. Here we examined the role of inhibition in cerebellar ventral paraflocculus (VPFL) function in awake macaques while they perform oculomotor tasks known to engage the VPFL. We used custom made carbon fiber multibarrel electrodes to examine the effect of injecting drugs that interrupt specific information pathways on the response of PCs. Our experimental approach has the advantage of examining cerebellar processing while the animal performs behavioral relevant computations. Our drug application affected a small portion of the VPFL (less than 1%), thus it has no effect on pursuit or saccade behavior. Moreover, we recorded the response of eye movement related mf with and without drug application and further verified that the efferent copy pathway is not affected by drug application. We found that injection of GABA-A receptor antagonist gabazine increased the gain of PCs to pursuit and VOR cancellation and made PCs responsive to saccades eye movements regardless of saccade direction. Injection of glycine blocker strychnine produced similar increases in gain than gabazine application, but had a lesser effect in PC saccade related responses. In contrast, injection of GABA-B receptor antagonist phaclofen reduced the response gain of PCs to pursuit. Overall, our result suggests that control of inhibition is a powerful mechanism to regulate the input-output gain of cerebellar cortex and spatial response properties of PCs. We hypothesize that the observed PC response gain changes during pursuit and VOR cancellation with application of gabazine and glycine are the result of modulating inhibition at the mf to granule cell synapses. In agreement, it has been shown that increasing inhibition reduces the gain of granule cells to mf stimulation. We also hypothesize that the effect over saccades responses is due to a different mechanism, perhaps involving molecular layer inhibition.
Disclosures: P.M. Blazquez: None. T.A. Yakusheva: None.

Poster
518. Cerebellum: Cortex and Nuclei Neurophysiology

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 518.12/S19

Topic: D.14. Cerebellum: Central Physiology

Support: ANR Blanc CerebComp (France)

Title: Temporal integration in an interneuron circuit model

Authors: *R. MAEX*, B. GUTKIN

Abstract: Inhibitory interneurons are integral parts of all brain circuits, and are commonly assumed to provide brisk control of spike timing, synchronization, circuit oscillations, etc. Theoretical studies have shown, however, that inhibitory circuits may also act as temporal integrators through a slow process of progressive disinhibition (Cannon, Robinson and Shamma, Biol. Cybern., 1983). We simulated a circuit of molecular layer interneurons, comprising a volume of about 100 (parallel-fibre axis) x 700 (sagittal) x 300 (depth) micrometers of cerebellar cortex. Each interneuron was implemented as a 22-compartment unit (an active soma and three passive dendrites, following Abrahamsson et al., Neuron, 2012). A population of 400 to 800 interneurons were interconnected through chemical (GABAA receptor) and electrical synapses, and activated by a pool of more than 10,000 parallel fibers firing Poisson spike trains. A narrow beam of parallel fibres conveyed the time-modulated, rectangular or sinewave stimulus. In the complete absence of inhibition, all interneurons spiked very fast with little variation across the network (mean ± s.d. 102 ± 10.4 spikes per second, CV 0.1), and they modulated their spike rates in-phase with the parallel-fibre stimulus. When the inhibition was strengthened, however, the overall spike rate decreased and could vary markedly across the network (13.5 ± 17.8, CV 1.3). Moreover, the most responsive interneurons spiked with a considerable phase lag of about 45 degrees at 0.2-0.5 Hz stimulation, corresponding to an integration time-constant of about 4 seconds. Three factors were able to enhance this integration time-constant: the strength of inhibition, the strength of electrical coupling, and the interneuron density. Electrical coupling depressed the overall spike rate, by spreading inhibition, but enhanced the response amplitude. In conclusion, the present simulation results indicate that networks of interneurons may act as low-pass filters, and they suggest that interneurons may be a component of the neural integrator thought to be distributed across the cerebellar - brainstem circuit.
Disclosures: R. Maex: None. B. Gutkin: None.

Poster

518. Cerebellum: Cortex and Nuclei Neurophysiology

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 518.13/S20

Topic: D.14. Cerebellum: Central Physiology

Support: M&B Program Regione SardegnaDR10-22462-1250/2011

Marie Curie Initial Training Network “NETT” Project No. 289146

Title: Encoding of virtual reality locomotion kinematics in vermis lobules V and VI of the mouse cerebellum

Authors: *S. MITOLO, T. MUZZU, S. R. SCHULTZ;
Imperial Col. London, London, United Kingdom

Abstract: The cerebellum has a well-established role in motor planning, execution and control. Recent imaging studies have shown that populations of cerebellar neurons are topically activated during locomotion (Ozden et al, PLoS ONE 7(8): e42650, 2012) and that optogenetic stimulation of Purkinje cells can affect the coordination of limb and trunk movements (Hoogland et al, Current Biology 25(9):1157-65, 2015). However to date it is still not well understood how the cerebellar network achieves movement control. We therefore aimed to characterize the activity of cerebellar neurons using high-density silicon electrode arrays in awake mice engaged in a locomotion task. We recorded from lobule V and VI of the cerebellar vermis of mice navigating in a virtual reality environment and we isolated more than 500 units. The cells were classified according to their electrophysiological properties (Hensbroek et al, J Neurosci Meth 232:173-80, 2014): the majority of the cells were identified as Purkinje cells, ~20% as Basket or Stellate cells and ~12% as granule cells. We then characterized the spiking activity of the cells in response to movement and found three main response profiles: speed-sensitive neurons, yaw-sensitive neurons and step cells. Speed-sensitive neurons could be further divided in positively tuned, negatively tuned and preferred speed neurons. Yaw-sensitive neurons modulated (50-200%) their activity with respect to the clockwise or anticlockwise direction. Step cells displayed a bursting activity synchronized with quick acceleration events caused by the animal stride. By recording simultaneously a large population of cerebellar neurons in a behaving animal we were able to encode locomotion-related parameters of cerebellar neurons. Further characterization of the
One of the most remarkable advances towards understanding cerebellar cortex computations in recent years has come from studies on the role of the cerebellar Nodulus and Uvula (NU, lobules IX and X) in spatial navigation in macaque monkeys. Macaque data suggest that NU are part of a neuronal circuit that transforms vestibular information, provided by our vestibular organs (semicircular canals and otolith organs), into heading direction (translation) and orientation with respect to gravity (tilt) using clearly defined mathematical operations. Most remarkably, genetically modified mice that lack postsynaptic LTD at parallel fiber to Purkinje cell synapses (L7-PKCI) have problems with spatial navigation when relying exclusively on internal cues (e.g. vestibular information). This finding strongly suggests that plasticity at parallel fiber to Purkinje cell synapses enables the structure to optimize computations and generate predictions of self motion necessary for path-integration. In this study we characterize the
response of NU Purkinje cells in wild-type alert mice using similar vestibular stimulation protocols to those we have previously employed in the macaque monkey. We tested the hypothesis that cerebellar LTD is necessary to enable the NU circuit to perform reliable estimations of heading direction (translation) and head orientation (tilt). Specifically, we characterized the response of NU Purkinje cells in the L7-PKCI transgenic mouse that expresses a protein kinase C (PKC) inhibitor in Purkinje cells, thus preventing normal PKC activity and LTD at parallel fiber to Purkinje cell synapses. We found that similar to macaques, NU Purkinje cells of wild-type alert mice carry the internal estimate of tilt and translation by combining transformed semicircular canal and otolith information. However, we found that mice and macaque NU Purkinje cells show differences in their dynamic response properties. Finally, our results suggest that plasticity at parallel fiber to PC synapses is essential to allow the temporal and spatial match of semicircular canal and otolith related information in NU. We conclude that some computational steps required estimating of tilt and translation occurred at parallel fiber to Purkinje cell and shaped by cerebellar plasticity.


Poster
518. Cerebellum: Cortex and Nuclei Neurophysiology

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 518.15/T2

Topic: D.14. Cerebellum: Central Physiology

Support: CIHR

Title: Selective encoding of unexpected head tilt by the deep cerebellar nuclei

Authors: *J. CARRIOT¹, M. JAMALI², J. X. BROOKS¹, K. E. CULLEN¹;
¹Physiol., ²McGill Univ., Montreal, QC, Canada

Abstract: The ability to distinguish sensory inputs that are a consequence of our own actions from those that result from changes in the external world is essential for perceptual stability and accurate motor control. We have previously shown that vestibular output neurons of the cerebellum, contributing to vestibulo-spinal reflexes and motion perception, robustly encode passively applied head rotation in the horizontal plane, while their responses are attenuated during comparable self-generated head motion. However, natural head movements are not restricted to one plane and generate more complex vestibular stimuli because of the presence of
the gravity. Therefore, we hypothesized that the brain uses an internal model that includes gravity to distinguish between self- versus externally- generated head motion. Interestingly, the ability to control posture and estimate unexpected self-motion depends strongly on the integration of vestibular, proprioceptive, and motor-related signals in cerebellum and is significantly disrupted in cerebellar patients. Thus, we tested our proposal by recording from single neurons from the rostral fastigial nuclei of the cerebellum in alert macaques during passive and active i) head-on-body tilts, as well as ii) head-on-body translations. We found that responses related to actively-generated tilts were significantly attenuated (relative to passively applied tilts). Moreover, this attenuation was comparable to that observed for active versus passive head translations (67 vs 74%, p>0.05). Taken together, our findings show that the neuronal coding of natural self-motion comprises an elegant computation of an internal model of active head motion that accounts for gravity.


Poster

518. Cerebellum: Cortex and Nuclei Neurophysiology

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 518.16/T3

Topic: D.14. Cerebellum: Central Physiology

Support: FOR 1847-A3 TH425/13-1

Title: Most caudal fastigial neurons of the monkey respond to saccades as well as smooth-pursuit eye movements

Authors: *Z.-P. SUN*1,2,3, P. W. DICKE4, P. THIER4;
1Hertie Inst. for Clin. Brain Research, Univ. of Tübingen, Tuebingen, Germany; 2Grad. Sch. of Neural and Behavioural Sci., 3Intl. Max Planck Res. Sch. for Cognitive and Systems Neuroscience, Univ. of Tübingen, Tuebingen, Germany; 4Dept. of Cognitive Neurol., Hertie Inst. for Clin. Brain Res., Tuebingen, Germany

Abstract: Saccades and smooth pursuit eye movements (SPEM) are two forms of goal directed eye movements with different kinematics, a difference that has contributed to the notion of largely segregated neuronal circuits for saccades and SPEM. Also previous work on the oculomotor role of the cerebellum seemed to suggest that distinct modules might be dedicated to the two types of goal directed movements, namely the flocculus/paraflocculus to SPEM and the oculomotor vermis (OMV) to saccades. However, first evidence requiring a modification of this
view became available already in the 80’s, when Suzuki and Keller (Suzuki & Keller, J Neurophysiol, 1988) described Purkinje cells (PC) in the OMV responsive to both saccades and smooth-pursuit, a characteristic that meanwhile turned out to be the rule rather than the exception (Smilgin, Dicke, & Thier, SFN abstract, 2012). The caudal fastigial nucleus (cFN) is a major target of PC axons originating from the OMV, serving as gateway for the oculomotor centers of the brainstem. Not surprisingly in view of the dependence of the cFN on the OMV, previous work has described both saccade- and SPEM-related single units in this nucleus, yet, without addressing the question if the two pools overlap. In other words, does the cFN continue the joint representation of SPEM and saccade-related signals found in the OMV or does it function as a track switch serving two independent pathways for SPEM and saccades starting here? To solve this question, we recorded 74 saccade-related neurons from the cFN. Of these, 28 were tested also for SPEM and, actually, 21 of them exhibited also clear SPEM-related activity. To estimate the sensitivities of these neurons for the major kinematic variables characterizing eye movements, we fitted their discharge with a linear combination of eye acceleration, velocity and position, independently for saccades and for SPEM. We found that the relative weights of the three variables were clearly different for saccades and SPEM. Saccade-related discharges were largely dominated by the position term without any difference between the subgroups of units tested for saccades only or for saccades as well as SPEM. On the other hand, SPEM-related responses were much more dependent on eye velocity. Sensitivity profiles for eye movement kinematics that are dependent on the type of eye movement executed with very similar properties as the one found in the cFN are also characteristic of OMV PCs. Hence, the cFN maintains a high degree of functional continuity with the OMV, offering saccade as well as SPEM-related signals to brainstem nuclei, hitherto usually assumed to be specific for saccades.

**Disclosures:**  
**Z. Sun:** None.  
**P.W. Dicke:** None.  
**P. Thier:** None.

**Poster**

**518. Cerebellum: Cortex and Nuclei Neurophysiology**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program/#Poster#: 518.17/T4

**Topic:** D.14. Cerebellum: Central Physiology

**Title:** Cerebellar nuclear neurons use time and rate coding to transmit Purkinje neuron pauses

**Authors:**  
*S. SUDHAKAR*1,2, B. TORBEN-NIELSEN1, E. DE SCHUTTER1,2;  
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Abstract: Neurons of the cerebellar nuclei convey the final output of the cerebellum to their targets in various parts of the brain. Within the cerebellum their direct upstream connections originate from Purkinje neurons. Purkinje neurons (PNs) of the cerebellar cortex have two distinct firing signatures: simple and complex spikes. The simple spikes are due to the intrinsic mechanisms of the cell and synaptic inputs coming through the parallel fibers and molecular layer interneurons (Gundappa-sulur, Schutter, & Bower, 1999; Palay & Chan-Palay, 1974; Raman & Bean, 1999). The PNs also emit complex spikes, which are due to strong excitation coming from the axons of the inferior olive cells, the climbing fibers (Palay & Chan-Palay, 1974). The simple spikes are highly regular intertwined with short pauses (Shin & De Schutter, 2006) while complex spikes occur sporadically and consist of burst of spikelets (Eccles, Llinas, & Sasaki, 1966). How can the cerebellar nuclei neurons downstream of the PNs make sense of this complex firing pattern? In this study we analyze how PN synchrony in the context of “pauses” in simple spikes affects different coding mechanisms of the neurons of the cerebellar nuclei. To this end, we use a computational model of a cerebellar nuclear neuron (Steuber, Schultheiss, Silver, De Schutter, & Jaeger, 2011) and synthetic PN spike trains. The coding mechanisms of cerebellar nuclear neuron can be broadly categorized as rate (Armstrong & Rawson, 1979; McDevitt, Ebner, & Bloedel, 1987) and time coding (Person & Raman, 2012). We define PN synchrony as synchronized Purkinje neuron pauses with either pause beginning or pause ending spikes precisely synchronized (Shin & De Schutter, 2006). With varying amount of pause synchrony for the above mentioned synchrony types, we analyzed its effects on time locking and rate coding in a nuclear cell. We find that Purkinje neuron synchrony is mainly represented as changes in the firing rate of cerebellar nuclei neurons. Pause ending type synchrony causes increased firing modulation. Pause beginning spikes produce better time locking of nuclear neurons compared to their pause ending counterparts for shorter length pauses but not for longer length pauses. We characterize the dependency of pause length and spike jitter on the coding strategy of the nuclear neuron. Additionally, we find that the rate of rebound responses in nuclear neurons after a synchronized pause is controlled by the firing rate of Purkinje neurons preceding it. We argue that these results lead to better understanding of how PN pause synchrony is processed in its target nuclear neuron.

Disclosures: S. Sudhakar: None. B. Torben-Nielsen: None. E. De Schutter: None.

Poster
518. Cerebellum: Cortex and Nuclei Neurophysiology

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 518.18/T5
A functional monosynaptic connection from the cerebellum to the ventral tegmental area

Authors: *C. H. CHEN¹, S. DORIZON², I. CARTA², K. KHODAKHAH²; ¹Dominick P. Purpura Dept. of Neurosci., ¹Albert Einstein Col. of Med., Bronx, NY

Abstract: The cerebellum is known for its coordination of movement, though it has also been implicated in non-motor behaviors. Converging evidence from neuroimaging and neuroanatomical studies suggest that the cerebellum contributes to a wide range of cognitive tasks, and dysfunction of the cerebellum may lead to cognitive disorders. Anatomical tracing studies suggest that the cerebellum has some inputs to the ventral tegmental area (VTA), a region where many dopaminergic pathways originate. Several of these pathways innervate areas that are known to be involved in cognitive processing, such as reward, and motivation. Therefore, we examined the connection between the cerebellum and the VTA. To test the functionality of this connection, we used optogenetics in mice to selectively excite cerebellar axons in the VTA while we monitored the activity of VTA neurons with extracellular recordings. Preliminary data show that the optical activation of cerebellar fibers in vivo increased the firing rate of neurons in VTA with a short latency of approximately a few milliseconds. To further investigate the nature of this connection, we optically stimulated cerebellar axons terminating in VTA in acutely prepared slices and found that it also resulted in short latency EPSCs in voltage-clamped VTA neurons. The sub-millisecond delay and the reversible blockade of these light-evoked currents by AMPA and kainate receptor antagonist CNQX in vitro suggest the existence of a monosynaptic excitatory pathway. Our data support the hypothesis that the cerebellum modifies the activity of the VTA and suggests that the cerebellum may play a role in modulation of the reward pathway. The behavioral consequences of manipulating this pathway are presented in another poster.

Synchrony is key. Olivocerebellar control of deep cerebellar nuclear (DCN) activity

**Authors:** *T. TANG*¹, C. Y. SUH¹, T. A. BLENKINSOP², E. J. LANG¹;
¹Neurosci. & Physiol., New York University, Sch. of Med., New York, NY; ²Mount Sinai, Sch. of Med., New York, NY

**Abstract:** Determining how DCN activity is modulated is central to understanding the signals the cerebellum sends to the rest of the CNS. Yet, the synaptic control of DCN activity is not well understood. For example, the large majority of synapses onto DCN cells are from Purkinje cells (PCs), yet, at least on a population level, simple spike and DCN firing rates are often found to co-modulate, suggesting that simple spikes have a relatively weak effect. Here, we explored the effect of complex spikes (CS) on DCN activity as an alternate control mechanism. CSs were recorded from PC arrays along with a single DCN neuron. CS-triggered correlograms of DCN activity were used to identify synaptically-connected PC-DCN cell pairs that formed narrow groups of 4-7 PCs. These correlograms showed that CS activity predominantly has an inhibitory effect on DCN activity. We tested whether the strength of the inhibitory effect of a CS varied as a function of its being synchronized with other cells in its group. The inhibitory effect was found to increase with the number of PCs in the group having synchronous CSs, such that isolated CSs produced little to no change in DCN activity, whereas highly synchronous CSs could cause a 60-80% drop. However, analysis of single trials showed the inhibitory effect was quite variable, even for the same synchrony level. A possible source of this variability is the variability of the CS waveform, as that may affect the number of axonically-propagated action potentials in a CS. We used signal variance to measure CS waveform, as this variance correlates with the number of spikelets in a CS; however, when synchrony is held constant, no consistent relationship between signal variance and the strength of the inhibitory action has been found thus far. Therefore, changes in waveform do not seem to alter the CS's action on the DCN significantly, and therefore could be modulated for other purposes, such as gating cortical plasticity, while changes in synchrony could be used to help shape DCN activity directly. Thus, by having distinct targets for their action, CS waveform and synchrony level, may allow the olivocerebellar system to perform its proposed roles in motor control and learning independently of each other.

**Disclosures:** T. Tang: None. C.Y. Suh: None. T.A. Blenkinsop: None. E.J. Lang: None.
**Title:** Cellular classification of fastigial/medial cerebellar nucleus output circuits

**Authors:** *H. FUJITA, T. KODAMA, S. DU LAC;* Johns Hopkins Univ., Baltimore, MD

**Abstract:** The midline cerebellum is involved in a wide range of functions including body posture, paced gait, eye movements, physiological homeostasis, attention, cognition, and emotion. The medial cerebellar (fastigial) nucleus (MCN), the sole output from the medial cerebellum, is responsible for this functional diversity, and is divided into rostral and caudal parts, which give rise descending and ascending projection, respectively. However, the critical diversity at a cellular level within the MCN is unclear. To molecularly classify individual MCN neurons and identify individual circuits, we quantitatively profiled expression of genes related to neurotransmitters, ion channels, and marker gene candidates for individual mouse MCN neurons. As a result of cluster analysis, sampled MCN neurons were functionally dichotomized by major quantitative differences in genes for ion channels as Kv3, which enables fast spiking. Further distinction of each group by qualitative or minor quantitative differences in other gene expression finally classified excitatory MCN neurons into four distinct groups. This classification was supported by distinct morphology of their neurons visualized with immunostaining for marker candidate proteins as Fzd7 and Spp1. Moreover, neurons in each group was localized to either rostral or caudal part of the MCN. For the ascending projection from the caudal MCN, retrograde tracing from VM thalamus or superior colliculus labeled distinct subset of MCN neurons, whose features were similar to those indicated in our molecular classification. Distinct projection targets for molecularly distinct neurons were so far also confirmed in the descending projection from the rostral MCN. With these results, we are investigating framework for each MCN function.

**Disclosures:** H. Fujita: None. T. Kodama: None. S. du Lac: None.

**Poster**

**Poster 518. Cerebellum: Cortex and Nuclei Neurophysiology**

**Location:** Hall A
Lobule-specific contribution of cerebellum to executive functions in mice

Authors: *A. M. BADURA*1,2, J. W. METZGER1, B. DEVERETT1, S. KOAY1, J. L. VERPEUT1, D. W. TANK1, S. S.-H. WANG1;

Abstract: Cerebellar lobules VI/VII and crus I/II form reciprocal loops with neocortical regions associated with executive functions (Wang, Kloth, & Badura 2014, Neuron 83:518–532), but the functional significance of those connections is not well explored. We hypothesized that the disruption of these cerebellar regions would lead to disruption in one or more executive domains: (1) social choice and behavioral inhibition, as measured using a 3-chamber test and an elevated plus maze; (2) cognitive flexibility as measured by the ability to change the preferred arm in a Y-maze swim task; and (3) working memory, as measured using a novel head-fixed visual evidence-integration task. Our results are consistent with specific roles for lobule VI in cognitive flexibility, and a role for crus II in behavioral inhibition. To achieve cell-type-specific inactivation of defined cerebellar regions, we took a pharmacogenetic approach using Designer Receptors Exclusively Activated by Designer Drugs (DREADDs). We used AAV8-hSyn-hM4D(Gi)-mCherry to express the inhibitory DREADD hM4D in molecular layer interneurons (MLIs). Inactivation of MLIs removes a major source of modulation of firing of Purkinje cells, which are inhibitory, putatively leading to net disinhibition of thalamus and neocortex. Mice were injected with the virus at 6 weeks of age and behavioral experiments followed 2 weeks later. Prior to each behavioral session mice were intraperitoneally injected with Clozapine-N-oxide (CNO), a DREADD agonist. In mice with DREADD expression in lobule VI, inactivation by CNO led to increased perseveration. Mice could learn to find an hidden platform in a swimming Y-maze, but showed impaired switching when the platform was moved to the other arm of the maze. The mice also showed prolonged individual bouts of grooming. In contrast, in a 3-chamber social choice between a novel mouse and a novel object, preferences were unchanged. We observed converse results in mice with DREADD expression in crus II, which is reciprocally connected with medial prefrontal cortex. CNO did not impair Y-maze reversal, but
did lead to increases in mouse-vs-object preference in the 3-chamber test, as well as increases in open-arm entries in an elevated-plus maze. These findings indicate a specific loss in behavioral inhibition. To probe executive function with greater temporal resolution, we are now testing mice using a head-fixed version of a working memory task (see abstract by Koay, Deverett et al., this meeting). In preliminary results, lobule VI-inactivated mice show reduced correct-performance trials and increased bias during evidence integration.


Poster

518. Cerebellum: Cortex and Nuclei Neurophysiology

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#/Poster#: 518.22/T9

Topic: D.14. Cerebellum: Central Physiology

Support: NS050808

NS079750

NS071665

Title: Cerebellar projections to VTA: a potential role for cerebellum in reward and social behavior

Authors: *I. CARTA¹, C. H. CHEN², S. DORIZAN², K. KHODAKHAH²;
²Dominick P. Purpura Dept. of Neurosci., ¹Albert Einstein Col. of Med., Bronx, NY

Abstract: Cerebellum is an important structure for movement. However, some of its projections terminate in brain areas that are not exclusively related to movement. Cerebellar fibers are found throughout the midbrain, including the ventral tegmental area (VTA). The VTA, through dopaminergic signaling, participates in reward, motivation and social related behaviors. In order to determine whether the cerebellar input to VTA can influence these complex behaviors we expressed either Channelrhodopsin2 or Archaerhodopsin (ArchT) in the deep cerebellar nuclei (DCN) of mice, and bilaterally implanted optical fibers above VTA. We then performed behavioral testing to assess reward seeking and sociability. In a simplified self-stimulation test we found that optical stimulation of cerebellar terminals in VTA is sufficient to encourage the mice to self-stimulate. Moreover, in the widely used three chamber social task, we found that the natural preference of mice for social contexts is reduced if stimulation is offered as an
alternative. The social preference is also decreased if the cerebellar input to VTA is silenced during the three chamber social task. Our data suggest that the cerebellum is an important upstream structure capable of shaping VTA responses to salient stimuli and might thus influence VTA function in processing reward and social behavior.


Poster

519. Gait: Muscle Activity, Exercise and Biomechanics

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 519.01/T10


Support: FRQS

NSERC

IRP

CIHR

Title: Speed modulation of locomotor gait in the adult mouse

Authors: *N. JOSSET1, M. LEMIEUX1, M. ROUSSEL1, S. COURAUD1, F. BREITZNER1,2;
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Abstract: Most quadrupeds exhibit a wide range of locomotor gaits from walk, to trot, to gallop. Interestingly, while the mouse can jump for 1 to 2 consecutive strides during flight reaction, there is little evidence that they can really gallop. Upon genetic manipulations of the spinal interneuronal circuit, some mutant mice can gallop with hindlimb synchronization, however, their wild-type littermates continue to walk even at high locomotor speed, thus raising some concerns as to whether the wild-type mouse can gallop and bound. Combining kinematic, angular excursion and electromyographic studies, we assessed locomotor gaits in 9 adult C57BL/6 mice (>3 weeks old) during steady-state locomotion at treadmill speeds ranging from 5 to 150 cm/s. Based on the symmetry/asymmetry of the gait (i.e. the presence or absence of hindlimb alternation) and the inter-limb coupling (e.g. between forelimbs, hindlimbs, as well as lateral limbs), we identified and characterized 2 symmetrical and 6 asymmetrical gaits during locomotion. Among symmetrical gaits, which were defined by hindlimb alternation, mice
displayed lateral walk and trot, but never diagonal walk or pace. Lateral walk was characterized by an anti-phase coupling in hindlimbs and forelimbs but an out-of-phase coupling in lateral limbs, while trot showed an anti-phase coupling in all pairs of limbs. Among asymmetrical gaits, two sub-types of locomotor gaits were identified according to either an in- or out-of-phase coupling in hindlimbs. Half-bound, full-bound, and hop were identified by hindlimb synchronization. Forelimbs were out-of-phase during half-bound and in-phase during full-bound, while they appeared disorganized during hop. Moreover, we also identified asymmetrical gaits with an out-of-phase coupling in hindlimbs such as in the rotary gallop, transverse gallop and asymmetrical walk. Using phase-frequency analysis, we found that mice exhibited a large spectrum of locomotor gaits across specific ranges of locomotor speed with episodes of lateral walk from 1 to 4 Hz (9% of gait events) and trot from 1 to 10 Hz (40.2%), all gallops above 7 Hz and up to 15 Hz, with the rotary gallop (3.6%), transverse gallop (5.9%), half-bound (10.8%), and full-bound (16.8%). Hop and asymmetrical walk showed a bimodal distribution with a peak at low speeds below 3 Hz and high speeds above 6 Hz, thus suggesting that they might be transition gaits. In summary, our study demonstrates that mice display a continuum of gaits as function of the locomotor frequency, ranging from walk to trot and then to gallop with various sub-types of gaits at the slowest and highest speeds.

**Disclosures:** N. Josset: None. M. Lemieux: None. M. Roussel: None. S. Couraud: None. F. Bretzner: None.

**Poster**

**519. Gait: Muscle Activity, Exercise and Biomechanics**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 519.02/T11

**Topic:** D.16. Posture and Gait

**Support:** NSF Grant DBI-RCN 1062052

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NIH Grant 5K12GM074869

**Title:** Motor control of forward accelerations versus steady swimming in bluegill sunfish

**Authors:** *M. A. SCHWALBE*, A. L. BODEN, T. N. WISE, V. VIKAS, E. D. TYTELL; Biol., Tufts Univ., Medford, MA
Abstract: Muscle can produce energy for movement, but can also change the effective stiffness or damping properties of a joint by co-contraction or eccentric contractions. Animals can shift muscles between these roles by changing relative timing of antagonist muscles. In particular, high speed behaviors require high muscle forces, but may also require an overall stiffer body because the reaction forces from the environment are also higher. How do animals control force production and stiffness modulation during high speed movements? We compared muscle activity during steady swimming and forward acceleration in bluegill sunfish (*Lepomis macrochirus*). We hypothesized that fish may increase the effective stiffness of their bodies by co-contracting left- and right-side muscles during acceleration, and may also activate more muscle during lengthening (eccentric contractions). To determine the muscle activity of a swimming fish, we implanted bipolar electromyographic electrodes in the superficial red axial muscle of bluegill sunfish and recorded muscle activation during forward accelerations and steady swimming between 0.5-2.5 body lengths/second. We used a new digital accelerometer to quantify the acceleration and 3D orientation of a fish’s body and also quantified swimming kinematics using high speed video. In forward accelerations, the muscles were active for a larger fraction of the tail beat cycle and came on at a different time compared to that in steady swimming. Further, duty cycles (percentage of strain cycle period) was greater in forward accelerations than in steady swimming. These results suggest that muscle on both sides of a fish’s body may co-contract during accelerations but not in steady swimming. Fishes likely control their effective body stiffness and enhance efficiency of both acceleration and steady swimming by shifting the timing and duration of muscle activity. By investigating the motor control of swimming in fish, we gain insights into how vertebrates control both high speed, high force movements and low speed, steady movements.

Disclosures: M.A. Schwalbe: None. A.L. Boden: None. T.N. Wise: None. V. Vikas: None. E.D. Tytell: None.

Poster

519. Gait: Muscle Activity, Exercise and Biomechanics

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 519.03/T12


Support: Institutional Startup fund from Temple University

ARO Grant 64929-EG

BBSRC Grant BB/J021504/1
Title: The Quakemill: A computer vision based actuated treadmill for rapid, precisely controlled mechanical perturbations of freely running animals

Authors: *A. VAHEDIPOUR, C. D. VALENTI, B. D. ROBERTSON, O. HAJI MAGHSOUDI, A. J. SPENCE;
Bioengineering, Temple Univ., Philadelphia, PA

Abstract: Locomotion is critical to survival and reproduction in most animals. As such it provides a powerful lens with which to view and subsequently understand the nervous system. Individuals often encounter obstacles and perturbations during locomotion. The mechanisms enabling recovery from these unexpected perturbations have not been fully described. A central difficulty is the integrated nature of this phenomenon. Feedforward neural commands, sensory feedback, and self-stabilizing aspects of the musculoskeletal system and whole body dynamics likely all contribute to recovery. A powerful paradigm with which to tackle this difficulty would be the application of external mechanical perturbations with and without simultaneous manipulation of the nervous system: so called “neuromechanical” perturbations. For example, these perturbations could target how gait is regulated to maintain stability after perturbation, or the function of sensory feedback in compensating for the unexpected. Here we present a computer vision controlled treadmill system capable of applying rapid, precisely timed, and spatially confined mechanical perturbations to freely running animals including mice and rats. We leverage robotic technologies developed by the Kod*Lab (University of Pennsylvania; G.C. Haynes et al., Proc. SPIE 8387, 2012), to create a fast, vertical displacement of the treadmill surface in closed-loop. We present data on the behavioral, gait, and postural kinematic response of C57BL/6 mice to sudden mechanical perturbations. We determined these responses with a multi-camera high-speed video system. Because the perturbations are triggered by a real-time feed of animal speed and position, confounding effects due to variation in these quantities were minimized. Combined with optogenetic manipulation of sensorimotor pathways, our long-term goal is to understand the integrated neuromechanical basis of robust legged locomotion. The system we present here represents a significant contribution to this aim. Through a better understanding of the neuromechanical basis of robust movement in the face of disturbances, we can improve outcomes for individuals with neurological disorders and musculoskeletal injuries, and provide inspiration for legged robots.


Poster

519. Gait: Muscle Activity, Exercise and Biomechanics

Location: Hall A
Title: Applying mediolateral pelvis force perturbation during treadmill training improves dynamic balance and overground walking in Children with Cerebral Palsy

Authors: *M. WU\textsuperscript{1,2}, J. KIM\textsuperscript{2}, D. J. GAEBLER-SPIRA\textsuperscript{2}, P. ARORA\textsuperscript{2};
\textsuperscript{1}Dept Physical Med. & Rehabil, Northwestern Univ., Chicago, IL; \textsuperscript{2}Sensor Motor Performance Program, Rehabil. Inst. of Chicago, Chicago, IL

Abstract: Cerebral palsy (CP) is the most prevalent physical disability originating in childhood with an incidence of 2-3 per 1,000 live births. Ninety percent of children with cerebral palsy have difficulty in walking. In addition, children with CP typically present with compromised balance control. Impaired balance control limits their walking capacity and negatively impacts their daily activities. Current standing balance training paradigms may improve standing balance but may have limited transfer to postural control during locomotion in children with CP. As a consequence, there is a need to develop a dynamic balance training paradigm for improving postural control during walking in children with CP. Previous study indicated that hip abductors/adductors plays a key role for maintaining lateral balance control during locomotion. In addition, muscle strength of hip abductors in children with CP was significantly less than in their able-bodies peers and appeared to be most closely related to walking function than any other lower-extremity muscle group. Thus, treatment techniques that focus on improving the motor control of the hip abductor/adductor muscles may improve lateral balance during locomotion in children with CP. We hypothesize that adding a lateral perturbation force to the pelvis during walking will increase the muscle activation of hip abductors/adductors, and repeated exposure of pelvis lateral perturbation loading will result in an improvement in dynamic balance and walking in children with CP. Ten children with CP (6 boys) have been recruited to participate in this study. The average age of these children was 10.2 ± 2.8 years old. The Gross Motor Function Measure ranged from I to III. A controlled force perturbation was applied to the pelvis in the mediolateral direction through a custom designed cable-driven robotic system while subjects walking on a treadmill. Muscle activities and kinematics of the pelvis and lower limbs were recorded during treadmill walking. In addition, standing balance, measured using a force-plate, and overground walking speeds were also measured before and after treadmill walking paired with pelvis force perturbation. Results indicated that pelvis force perturbation induced an enhanced muscle activities of hip abductors/adductors during treadmill walking in children with CP. In addition, overground walking speed and step width also improved in children with cerebral palsy following one session treadmill training paired with pelvis force perturbation.
These techniques could be used as an intervention to improve the dynamic balance and walking function in children with cerebral palsy.

**Disclosures:** M. Wu: None. J. Kim: None. D.J. Gaebler-Spira: None. P. Arora: None.

**Poster**

**519. Gait: Muscle Activity, Exercise and Biomechanics**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 519.05/T14

**Topic:** D.16. Posture and Gait

**Support:** NIH R01 Grant HD081274

Wings for Life Clinical Trial Award

Georgia Institute of Technology Institute for Bioengineering and Biosciences Seed Grant

**Title:** Constraints on stance-phase force production and muscle coordination during overground walking in persons with chronic incomplete spinal cord injury

**Authors:** *H. B. HAYES, S. L. TIRADO, R. D. TRUMBOWER; Dept of Rehabil. Med., Emory Univ., Atlanta, GA

**Abstract:** Persons with incomplete spinal cord (iSCI) struggle with community ambulation. They exhibit decreased walking speed, reliance on assistive devices (ADs), and increased fall risk, suggesting forces for propulsion, weight support, and stability are impaired. Muscle weakness and inappropriate muscle coordination may impede force production, but how altered muscle coordination constrains force production remains unknown. The purpose of this study is to investigate constraints on force production during the stance phase of overground walking in persons with iSCI and the underlying muscle coordination deficits. We hypothesize that persons with iSCI exert smaller forces than age-matched able-bodied controls (AB) walking at matched cadences and fail to modulate forces with increased cadence. We hypothesize that differences in iSCI forces coincide with maximum muscle co-activity. To test our hypotheses, persons with chronic iSCI and AB walked overground at self-selected and fast cadences. AB also walked at the matched cadences and ADs of iSCIs. Ground reaction forces generated by the more impaired limb were recorded along with surface electromyography of 12 leg muscles. Non-negative matrix factorization identified groups of consistently co-activated muscles, or modules, and their activation across stance. Persons with iSCI exhibit reduced forces due to muscle co-activity and weak extensor activation. iSCI persons exert significantly lower braking and propulsive impulses.
in the fore-aft axis and lower peak vertical forces during late stance compared to AB walking at matched cadences and ADs. Reduced braking aligns with activation of co-activity modules composed of muscles across the ankle, knee, and hip. Reduction in late-stance propulsive forces scale with lower extremity motor scores and align with weak plantarflexion module activation. Force reductions are exaggerated with ADs, even compared to AB walking at the same cadences and ADs. Finally, when walking at faster cadences, iSCI show limited force modulation as compared to AB. Those with ADs even show opposite modulation compared to AB, suggesting force impairments and reliance on ADs increase with effort. Consistent with our hypotheses, ground reaction forces are constrained during overground walking in persons with chronic iSCI resulting from both muscle weakness and inappropriate co-activity. When using ADs, walking faster as often sought in rehabilitation, may actually exaggerate muscle dis-coordination and compensation. Understanding these force and coordination impairments is vital for targeted rehabilitation strategies to improve overground walking ability in chronic iSCI.


Poster

519. Gait: Muscle Activity, Exercise and Biomechanics

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 519.06/T15


Support: NIH NINDS NS086973

Title: Muscle actions on task performance and joint integrity in the rat hindlimb


Abstract: Although the neural control of movement is usually considered in terms of the control of task relevant variables, neural systems might also control the state of other variables that have a less visible impact upon behavior. In particular, we are examining the hypothesis that the nervous system actively monitors and regulates the state of internal joint variables in order to minimize excessive stresses and strains on joint cartilage and ligaments. We are examining these issues in the rat, evaluating whether the nervous system activates hindlimb muscles to control both task performance (i.e. locomotion) and joint integrity (i.e. knee joint structures). We
examined the biomechanics of the patellofemoral joint in the rat and its control by quadriceps muscles. In acute experiments we measured the kinetics and kinematics of hindlimb movements evoked by stimulation of the three main quadriceps muscles in the rat: rectus femoris (RF), vastus lateralis (VL), and vastus medialis (VM). The pelvis was held in place using bone screws and the distal tibia attached to a 6 axis force transducer. Retroreflective markers were rigidly attached to the patella and femur and their 3D position was tracked. Each quadriceps muscle was then stimulated and the evoked isometric forces and kinematic motions were recorded. We found that the isometric forces evoked by VM and VL were very similar to one another (3D forces differing by ~5 degrees). Consistent with this similarity, there was minimal mediolateral movement of the patella in the femoral groove produced by stimulation of any of quadriceps muscle. However, each muscle produced opposing rotations of the patella within the groove, suggesting that these muscles exerted opposing torques on the patella. Additional experiments confirmed these results, showing that VM and VL evoked similar hindlimb trajectories when the limb was allowed to move freely but that they evoked opposing mediolateral forces on the patella when the force transducer was attached directly to the patella. These biomechanical experiments allow us to make strong predictions on neural control strategies across a range of perturbations and thereby to evaluate how the nervous system controls both task and joint variables during behavior.


Poster

519. Gait: Muscle Activity, Exercise and Biomechanics

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 519.07/T16


Title: Feedback and feedforward control during walking in individuals with chronic ankle instability

Authors: *S.-C. YEN¹, M. B. CORKERY¹, A. DONOHOE¹, M. GROGAN¹, Y.-N. WU²; ¹Dept. of Physical Therapy, Northeastern Univ., Boston, MA; ²Univ. of Massachusetts Lowell, Lowell, MA
Abstract: Lateral ankle sprains (inversion injuries) are a common injury encountered in physical activities. Up to 70% of individuals who have an ankle sprain eventually develop chronic ankle instability (CAI), which is characterized by residual pain, weakness, and most problematically, recurrent ankle sprains. The cause of recurrent ankle sprains remains unclear, but has been linked to impaired feedback and feedforward control of the ankle joint. The purpose of this study was to compare the feedback and feedforward control between healthy individuals and individuals with CAI when they walk with a perturbation inverting the ankle joint. We hypothesized that the feedback and feedforward control are different between the two groups. Twelve young subjects with CAI and 12 healthy subjects participated in this study. They were asked to walk on a treadmill in 3 sequential periods. The first period was the baseline period where subjects walked normally on a treadmill without any intervention for 1 minute. The second period was the adaptation period where subjects walked with a 1-lb weight placed on the dorsal-lateral side of the foot (inversion perturbation) for 5 minutes. Subjects’ reaction to the weight can generate insight into the feedback control strategy. The third period was the post adaptation period where the weight was removed while subjects continued to walk for 1 minute. Subjects’ kinematic change detected in this period (i.e., aftereffect) can generate insight into the feedforward control strategy. Subjects’ ankle kinematics was recorded and calculated using 3D motion capture system. The kinematic analysis was focused on 30 ms before and after heel contact where ankle sprains are most likely to occur. We found that healthy individuals increased ankle eversion to counteract the perturbation during both early and late adaptation period. After the weight was removed during the post adaptation period, they showed a significant aftereffect consisting of increased ankle eversion. In contrast, subjects with CAI only increased ankle eversion in the early adaptation period. In the late adaptation period, the ankle angle in the frontal plane returned to the baseline. After the weight was removed, they showed no significant aftereffect. The results suggest that CAI is associated with changes in feedback and feedforward control of the ankle joint during walking. The results may help explain why individuals with CAI recurrently sprain the ankle during daily walking.


Poster

519. Gait: Muscle Activity, Exercise and Biomechanics

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 519.08/T17

Support: NSERC
FRQS

Title: Locomotor control in mutant mice lacking DSCAM: a kinematic and EMG study

Authors: *M. LEMIEUX*¹, F. BRETZNER²;
¹Ctr. De Recherche Du CHU De Québec, Quebec, QC, Canada; ²Ctr. De Recherche Du CHU De Québec, Québec, QC, Canada

Abstract: Quadrupedal locomotion is an adaptable behavior requiring coordination within each limb, between the left and the right side of the pectoral and pelvic girdles and between girdles. The proper orchestration of spinal networks operating at each level depends on local and long-range connectivity that is genetically determined during development. Down Syndrome Cell Adhesion Molecule (DSCAM) is an immunoglobulin that has been involved in several neurodevelopmental processes. Interestingly, mice lacking DSCAM stood with hyperextended limbs and a flexed back at rest reminiscent of the scaredy cat posture. However, little is known about their functional locomotor and postural impairments. Combining kinematic and electrophysiologic studies in mice, we have investigated the impact of a spontaneous mutation of DSCAM (DSCAM2J) on locomotor control. Reflective markers were placed on hindlimbs joints to analyze limb movements. Mice were videotaped during treadmill locomotion at steady speeds ranging from 0.05 to 0.9 m/s. Based on individual strides, we identified different locomotor gaits: asymmetrical walk (out of phase hindlimbs), lateral walk (anti-phase hindlimbs, low homolateral couplet), trot (anti-phase forelimbs, hindlimbs and homolateral limbs), hop (in-phase hindlimbs) or pace (anti-phase hindlimbs and in-phase homolateral limbs). WT mice exhibited predominantly asymmetrical walk at very low speed, lateral walk at low to moderate speed and trot at higher speeds. In contrast, DSCAM2J mice predominantly used asymmetrical walk at low and moderate speeds and lateral walk instead of trot at higher speeds. DSCAM2J mice also hopped at moderate speeds and paced at all speeds whereas WT mice used these two gaits only at low speed. DSCAM2J were more frequently supported on two limbs rather than on three limbs like WT mice. At the intralimb level, DSCAM2J mice generally presented a larger stride height and length than WT mice. We found a larger and more variable angular excursion for proximal and intermediate joints for DSCAM2J mice. The occurrence of the hyperextended posture of DSCAM2J typically seen at rest and during hops decreased during asymmetrical walk and was rarely observed during lateral walk, trot and pace. Our electrophysiologic recordings showed that the amplitude of electromyographic responses and the coordination between flexor and extensor muscles were impaired at low speed but improved at high speed. We conclude that the DSCAM mutation alters the output of neural networks involved in intralimb and interlimbs coordination during locomotion.

Disclosures: M. Lemieux: None. F. Bretzner: None.
Abstract: A close relation between hippocampus and locomotion has been described by several authors. After a penetrating injury in hippocampus, tamoxifen promotes the recovery of the hindlimb kinematics and the brain cortex electrical activity. The present experiments were performed in 2 months old female rats to determine the kinematic changes after a penetrating injury (PI) in the left hippocampus. Hindlimb and forelimb kinematics were studied: before, 7, 15 and 30 DPI. A total of 13 subjects were studied (cortex group n=3, hippocampal non-treated group n=5 and hippocampal tamoxifen group n=5). After 30 days the subjects were anesthetized and intracardially perfused to obtain the hippocampal tissue and cell counts were performed. We observed kinematic changes in the ipsilateral and contralateral hindlimbs as well as in the ipsilateral forelimb due to the hippocampal lesion, which were not present in the cortex group. Tamoxifen effect was noticeable at 7 and 30 DPI, reducing in a significant manner the kinematic changes present in the non-treated group. Tamoxifen favored the preservation of the hippocampal pyramidal neurons on the CA1 region, this could be related to the better kinematic outcome of the tamoxifen treated group.
Title: Predictive and one-step-behind algorithms for self-paced split-belt treadmill

Abstract: Trauma to the nervous system or even to the musculoskeletal system generally results in asymmetric movement and adaptations in animals, including humans. Common rehabilitation of asymmetric gait is locomotor training that may involve walking on an instrumented split-belt treadmill to accommodate the interlimb asymmetry. The rational of this study was to develop a self-paced split-belt treadmill that adapts to individual performance with independent belt control without the use of motion capture. Using real-time analysis of ground reaction forces (GRF) we extracted the following step parameters: swing and stance phase durations, stride length, and ground contact on the treadmill for each leg. The speed of each leg was calculated at phase transitions (swing to stance & stance to swing) detected by thresholding vertical GRF components. The limb speed was then calculated as a ratio of stride length divided by the cycle duration, where the stride length was a sum of the difference in interstride contact positions and the integrated speed signal over the past cycle duration. Two components, based on the interstride differences in gait phases, were also added to reduce uncontrolled belt speed drift. The resulting signal was then used in a closed-loop one-step-behind controller to update independently the speed of each treadmill belt. In addition, we have augmented this controller with an acceleration-based prediction. These two types of controllers were tested during locomotion over a range of velocities to evaluate their performance. Subjects were instructed to walk symmetrically; therefore, the interlimb speed difference could be used to access the overall controllability of the treadmill. Both algorithms were sufficiently robust to allow self-paced locomotion at desired pace and speed, with the predictive algorithm showing the best performance.

Disclosures: M. Boots: None. S. Yakovenko: None.

Poster

519. Gait: Muscle Activity, Exercise and Biomechanics

Location: Hall A
Title: Auditory cue modifies the fractal dynamics of human gait during treadmill walking

Authors: *K. MASANI*, H. ROUHANI, M. O. ABE, K. NAKAZAWA, D. NOZAKI;

1Toronto Rehab Inst., Toronto, ON, Canada; 2Inst. of Biomaterials and Biomed. Engin., Univ. of Toronto, Toronto, ON, Canada; 3Grad. Sch. of Educ., Hokkaido Univ., Sapporo, Japan; 4Dept. of Life Sci., 5Grad. Sch. of Educ., The Univ. of Tokyo, Tokyo, Japan

Abstract: Stride interval fluctuation during walking has unique dynamics called long-range correlation or fractal dynamics. It was described that the fractal dynamics disappear when one walks with rhythmic auditory cues, such as metronome sounds. Here we quantitatively investigated the dynamics of the stride interval fluctuation during treadmill walking with auditory cues to reveal how the fractal dynamics is modified by the cue. Ten healthy subjects (18 - 24 yrs) participated in this study. Each subject was requested to walk on a treadmill with his/her natural cadence and speed (natural walking). The average cadence was calculated in the first trial, and then the subject was asked to walk with the same speed while a metronome sound with average cadence was added (metronome walking). Detrended fluctuation analysis (DFA) was performed to examine the fractal dynamics of each gait condition. Agreeing with previous studies, the DFA plot for metronome walking showed a curve at around a window period of 30 steps. Thus, we calculated the slope of DFA plot before (1st period) and after (2nd period) the window period of 30 steps, separately. Further, we performed a simulation study based on the assumption that stride interval fluctuation during metronome walking is a summation of innate fractal rhythm and an error of the current rhythm from the metronome sound. The alpha value for natural walking was 0.96±0.09, suggesting that natural walking has fractal dynamics even with a constrained speed (i.e., walking on a treadmill), which agrees with previous studies. In contrast, metronome walking showed alpha value of 0.37±0.16 for the entire range, suggesting that long-range correlation disappeared. However, 0.21±0.21 for the 1st and 2nd periods, respectively. These alpha values were different from each other, indicating that the DFA plot for the metronome walking was curved with a convex shape. In the simulation study, we found that the simulated stride interval fluctuation showed a curved DFA plot very similar to the one in the experiment. This result suggests that fractal dynamics were preserved even during metronome walking while an error correction based on the previous step modifies the stride interval.

Title: Use of inertial sensors for determining kinematic characteristics of infant leg movement

Authors: *I. A. TRUJILLO PRIEGO*, B. A. SMITH;

Dept. of Biokinesiology, USC Div. of Biokinesiology and Physical Therap, Los Angeles, CA; USC Div. of Biokinesiology and Physical Therapy, Univ. Of Southern California, Los Angeles, CA

Abstract: Our overall goal is to use data from inertial sensors to determine the quantity, type and quality of infants’ leg movements performed across a full-day measurement for further use in the differentiation of infants with typical, delayed or impaired neuromotor development. Here we describe an algorithm to calculate kinematic characteristics of leg movements. Inertial sensor data were collected from 12 infants with typical development for a period of 8-13 hours per day. There were 2 months between visits and a total of 3 visits per infant. An inertial sensor was attached to each leg, recording simultaneously accelerometer and gyroscope measurements at 20Hz. In previous work, we developed and validated a threshold-based algorithm where each pause or change of direction of the limb is counted as a discrete movement. Here we determined the duration, average acceleration, and peak acceleration of each movement. The duration of each movement was computed by counting the number of samples when the acceleration magnitude was above baseline until it crossed the baseline for a second time. Consequently, acceleration magnitude was obtained for each of these samples and average acceleration and peak acceleration of each movement was calculated. Infants produced average movement durations that ranged from 0.23 to 0.33 seconds per movement, with average accelerations ranging from 1.59 to 3.88 m/s² and average peak accelerations from 3.10 to 8.83 m/s². Mean (M) and standard deviation (SD) of movement duration (D), average acceleration (AA) and peak acceleration (PA) for each infant at each visit, for right and left legs, are shown in Table 1. Our results showed that there is a range of leg movement duration and acceleration values produced by infants across visits. Future work will focus on the analysis of movement features based on
the developmental level of infants and identification of the differences between infants with typical, delayed or impaired neuromotor development.

Table 1: Kinematic Characteristics of Leg Movements, by Infant by Visit

<table>
<thead>
<tr>
<th>Infant</th>
<th>Visit</th>
<th>Age (months)</th>
<th>Movement Duration, Right Leg (s) (M ± SD)</th>
<th>Movement Duration, Left Leg (s) (M ± SD)</th>
<th>Average Acceleration, Right Leg (m/s²) (M ± SD)</th>
<th>Average Acceleration, Left Leg (m/s²) (M ± SD)</th>
<th>Average Peak Acceleration, Right Leg (m/s²) (M ± SD)</th>
<th>Average Peak Acceleration, Left Leg (m/s²) (M ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1</td>
<td>6</td>
<td>0.30 (0.15) 0.2</td>
<td>0.30 (0.15) 0.2</td>
<td>1.93 (1.33) 2.0</td>
<td>1.91 (1.38) 2.1</td>
<td>3.84 (3.13) 4.1</td>
<td>3.81 (3.09) 4.2</td>
</tr>
<tr>
<td>A</td>
<td>2</td>
<td>8</td>
<td>0.15 (0.14) 0.2</td>
<td>0.16 (0.14) 0.2</td>
<td>1.52 (1.45) 2.1</td>
<td>1.56 (1.41) 2.1</td>
<td>4.15 (3.41) 4.8</td>
<td>4.05 (3.42) 4.3</td>
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<tr>
<td>B</td>
<td>3</td>
<td>10</td>
<td>0.28 (0.13) 0.2</td>
<td>0.28 (0.14) 0.2</td>
<td>1.42 (1.45) 2.1</td>
<td>1.41 (1.40) 2.1</td>
<td>4.72 (3.85) 4.8</td>
<td>4.65 (3.86) 4.4</td>
</tr>
<tr>
<td>B</td>
<td>4</td>
<td>12</td>
<td>0.14 (0.27) 0.1</td>
<td>0.13 (0.26) 0.1</td>
<td>1.57 (1.32) 2.0</td>
<td>1.77 (1.28) 2.0</td>
<td>3.25 (3.08) 4.5</td>
<td>3.37 (3.10) 4.7</td>
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<td>5</td>
<td>15</td>
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<td>0.21 (0.15) 0.2</td>
<td>1.21 (1.54) 2.1</td>
<td>1.32 (1.40) 2.1</td>
<td>2.62 (2.52) 3.5</td>
<td>2.65 (2.49) 3.6</td>
</tr>
<tr>
<td>C</td>
<td>6</td>
<td>17</td>
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<td>0.14 (0.28) 0.1</td>
<td>1.57 (1.28) 2.0</td>
<td>1.47 (1.28) 2.0</td>
<td>3.08 (2.93) 4.0</td>
<td>3.09 (2.87) 4.1</td>
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<tr>
<td>D</td>
<td>7</td>
<td>21</td>
<td>0.12 (0.14) 0.2</td>
<td>0.13 (0.14) 0.2</td>
<td>1.42 (1.52) 2.0</td>
<td>1.42 (1.45) 2.0</td>
<td>2.51 (2.39) 3.6</td>
<td>2.50 (2.33) 3.7</td>
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<tr>
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<td>23</td>
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<td>0.14 (0.28) 0.1</td>
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<td>1.47 (1.28) 2.0</td>
<td>3.08 (2.93) 4.0</td>
<td>3.09 (2.87) 4.1</td>
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<tr>
<td>D</td>
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<td>27</td>
<td>0.32 (0.13) 0.2</td>
<td>0.33 (0.14) 0.2</td>
<td>1.51 (1.23) 2.0</td>
<td>1.51 (1.23) 2.0</td>
<td>3.08 (2.93) 4.0</td>
<td>3.09 (2.87) 4.1</td>
</tr>
<tr>
<td>E</td>
<td>10</td>
<td>31</td>
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<td>0.14 (0.28) 0.1</td>
<td>1.42 (1.52) 2.0</td>
<td>1.42 (1.45) 2.0</td>
<td>2.51 (2.39) 3.6</td>
<td>2.50 (2.33) 3.7</td>
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<tr>
<td>E</td>
<td>11</td>
<td>33</td>
<td>0.14 (0.27) 0.1</td>
<td>0.14 (0.28) 0.1</td>
<td>1.42 (1.52) 2.0</td>
<td>1.42 (1.45) 2.0</td>
<td>2.51 (2.39) 3.6</td>
<td>2.50 (2.33) 3.7</td>
</tr>
<tr>
<td>F</td>
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<td>35</td>
<td>0.14 (0.27) 0.1</td>
<td>0.14 (0.28) 0.1</td>
<td>1.42 (1.52) 2.0</td>
<td>1.42 (1.45) 2.0</td>
<td>2.51 (2.39) 3.6</td>
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Disclosures: I.A. Trujillo Priego: None. B.A. Smith: None.

Poster

519. Gait: Muscle Activity, Exercise and Biomechanics

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 519.13/U2


Support: Mizuno Sports Promotion Foundation (2014)

Title: Stepwise shifts in the set of modules for human locomotion with speed change

Authors: *H. YOKOYAMA¹, T. OGAWA¹, N. KAWASHIMA², K. NAKAZAWA¹;
¹The Univ. of Tokyo, Tokyo, Japan; ²Res. Inst. of Natl. Rehabil. Ctr. for Persons with Disabilities, Saitama, Japan

Abstract: Spinal neural circuits can generate locomotor movements in a wide-range of speeds. Recent studies in vertebrates have revealed that different spinal circuits are activated depending on locomotor speeds. Although there are a few behavior-based studies, it is unknown whether different spinal neural circuits are responsible for generating different speeds of locomotor movements in human. To answer this question, factorization algorithms were used for extracting
locomotor modules: functional units implemented in spinal neural circuits. By applying this technique, we tested the hypothesis that human locomotor modules shift with changing speed. Eight healthy adults and eight collegiate runners walked or ran on a treadmill linearly increasing speed from 0.3 to 4.3 m/s and from 0.3 to 5.0 m/s, respectively (ramp speed condition, acceleration of the belt was set to 0.01 m/s²). Subjects were instructed to either walk or run on the basis of their preference under the given speed. Surface EMGs were recorded from 16 muscles on the one side of the trunk and leg. Locomotor modules were extracted from the EMGs by using the non-negative matrix factorization algorithm. Then, commonality of modules among subjects and speeds was evaluated based on the correlation coefficients. In addition, to evaluate the influence of the differences of modules among speeds on generation of muscle activity, the combined method of EMG reconstruction method and cluster analysis were executed. Assuming that similar module sets generate similar EMG activity, EMG activities across all speeds were repeatedly reconstructed by using modules at each speed. After that, the characteristics of the reconstruction quality were grouped by cluster analysis. The results showed that the number of modules and the components of modules were different among speeds. Further, the characteristics of EMG reconstruction were different between walking and running. Then, those of walking and running were respectively divided into two subgroups: slow and fast one. Namely, discrete modules are activated with speed change. These results strongly confirmed our working hypotheses, and the results clearly support the view that neural circuits for human locomotion shift in a speed-dependent manner.

Disclosures: H. Yokoyama: None. T. Ogawa: None. N. Kawashima: None. K. Nakazawa: None.

Poster

519. Gait: Muscle Activity, Exercise and Biomechanics

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#/Poster#: 519.14/U3


Support: Ohio Department of Development, TECH 09-001

Title: Knee joint impedance optimizations for design of transfemoral prostheses

Authors: *H. ARGUNSAH BAYRAM¹, M. B. BAYRAM¹, B. L. DAVIS²;
¹medical engineering, Acibadem Univ., istanbul, Turkey; ²Biomed. Engin., Univ. of Akron, Akron, OH
Abstract: Knee joint impedance describes the knee resistance to rotations and possible buckling. It is defined as the ratio of the kinetics of the joint, (i.e., the torque applied to the knee to rotate it), and its kinematics (i.e., the angular velocity at the knee) during static and dynamic situations. Muscles are the key impedance suppliers. In exploring the time intervals, when the energy is harvested and dissipated at the knee, impedance analysis is extremely important. A prosthetic knee, which can store energy in the spring when it is available and then release the appropriate amount of it at the appropriate time during the daily activities, could potentially greatly enhance ambulation. Information regarding impedance of knee is important for understanding the energy flow at the knee and designing energy regenerative prostheses. This study examined knee mechanics in 12 healthy subjects to explore impedance in the energy harvest and return phases during the activities of daily living (ADL’s). The reason for assessing different ADL’s is because it is possible that impedance of the knee is dependent on the musculoskeletal task being performed. During the energy return phases, high impedance is obtained due to the resistance to motion. Therefore, the stored energy is absorbed at the knee during these periods. Conversely, during the energy harvest phases, low impedance is obtained due to the knee’s intention to assist to motion. Thus, energy is harvested during these periods. These findings are helpful for identifying prosthetics design strategies, so that by using modern computational tools, prosthetic knees could be simulated even before initiating the hardware design process. Impedance is calculated by performing a regression of sagittal plane knee flexion moment and knee angular velocity curves for the energy harvest and return phase intervals of the activities. The slopes of the torque versus angular velocity regressions are defined as the impedance. Energy return phase impedance varied between -0.005 Nm s/kg deg. and 0.047 Nm s/kg deg., and energy harvest phase impedance varied between -0.002 Nm s/kg deg. and 0.004 Nm s/kg deg. The erratic impedance values indicated the difficulty of achieving high efficiency during all activities in a simple mechanism.

Disclosures: H. Argunsah Bayram: None. M.B. Bayram: None. B.L. Davis: None.

Poster

519. Gait: Muscle Activity, Exercise and Biomechanics

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 519.15/U4


Title: Reducing gait asymmetry after stroke with strength training
**Authors:** *J. W. STINEAR, A. J. C. MCMORLAND, M.-J. JEON;*
Univ. of Auckland, Auckland, New Zealand

**Abstract:** Hemiparesis is a common sequel to stroke that typically results in an asymmetrical gait pattern. We designed an experiment to test the hypothesis that plantarflexor (PF) and dorsiflexor (DF) strength training reduces gait asymmetry. Fifteen chronic stroke patients with a walking velocity between 0.6 and 0.8 m/s conducted strength training using Therabands three days a week over six weeks. Two pre-intervention measures of PF and DF strength were taken 2-4 weeks apart. Timing of muscle activity was also assessed using surface electromyography (EMG) during walking, while a Vicon motion capture system recorded patients’ kinematic and kinetic data. One set of post-intervention measures was taken immediately after the training period followed by another six weeks later. The study will conclude at the end of May, 2015. Preliminary results are as follows. Strength training improved paretic limb PF strength by approximately 45%. Only minimal gains in DF strength were observed. There was a trend for paretic limb step time to decrease by 9%, and double support time to decrease by 15% when the paretic limb trailed; this was also accompanied by a 19% reduction in the duration of paretic limb PF muscle activity. These data partially support our hypothesis, and suggest that after stroke, strength training for muscles activating the ankle reduces some measures of gait asymmetry.

**Disclosures:** J.W. Stinear: None. A.J.C. McMorland: None. M. Jeon: None.

**Poster**

519. Gait: Muscle Activity, Exercise and Biomechanics

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 519.16/U5

**Topic:** D.16. Posture and Gait

**Support:** NIDRR H133B031127

**Title:** Alterations in sagittal and frontal gait kinematics in patients with subacute stroke following high-intensity stepping training

**Authors:** *T. G. HORNBY¹², G. MAHTANI³, M. CONNOLLY³, C. HOLLERAN³, P. HENNESSY³, J. WOODWARD³;
¹Dept. of Physical Therapy, Univ. of Illinois at Chicago, Chicago, IL; ²Sensory Motor Performance Program, ³Rehabil. Inst. of Chicago, Chicago, IL*
Abstract: Background: Development and reinforcement of abnormal gait kinematics is a primary concern for therapist treating patients post-stroke. A common goal of locomotor training strategies is to approximate “normal” kinematic patterns and walking speeds, although the negative outcomes of robotic and therapist-assisted gait training strategies raise questions regarding the importance of this strategy. Recent strategies that focus on providing large amounts of high-intensity stepping practice in variable contexts have not focused on minimizing abnormal gait patterns, but have resulted in substantial improvements in clinical locomotor outcomes. Allowing patients to explore different kinematic solutions to successfully complete stepping tasks could lead to development of compensatory gait strategies, although the resultant kinematic outcomes have not been well described. The present study investigated changes in sagittal and frontal plane kinematics following high intensity locomotor training in patients < 6 mo post-stroke as compared to conventional strategies. Methods: The present data represents a subgroup analysis of kinematic outcomes from two separate, completed studies (Holleran 2014, 2015). Eligible subjects included those who ambulated at overground gait speeds < 0.90 m/s. Subjects completed up to 40 1-hr sessions of high intensity stepping training in variable contexts, with focus only on completing walking tasks. The control group completed up to 40 sessions of low-intensity variable task training. Kinematic data collection was performed during graded treadmill testing (start at 0.1 m/s, increased by 0.1 m/s every 2 minutes) at pre- and post-training (22 experimental, 13 controls). Data were collected at 1000 Hz with an 8 camera motion capture system with 32 lower limb, reflective markers (modified Cleveland Clinic set). Primary outcomes included sagittal plane joint excursions and frontal plane hip hiking/circumduction. Results: Subjects in the experimental group achieved greater increases in peak speeds (0.56 vs 0.13 m/s), cadence, paretic step length and sagittal plane hip excursion than the control group. Frontal plane hip hiking was significantly increased at peak speeds post-training in the experimental vs control group (3.0 vs 0.3 deg). Increases in frontal plane changes were related to baseline impairments (Fugl-Meyer). Conclusions: Significant but small changes in frontal plane kinematic strategies were observed following high-intensity stepping training in variable contexts. Such changes may be due to initial impairments and lack of residual motor function to successfully retrain normal gait patterns.


Poster

519. Gait: Muscle Activity, Exercise and Biomechanics

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 519.17/U6
Visual feedback allows altered muscle phasing during pedaling for both the paretic and non-paretic leg following stroke

Authors: *C. H. MULLENS¹, D. A. BROWN²;
¹Physical Therapy and Human Movement Sci., Northwestern Univ., Chicago, IL; ²Univ. of Alabama, Birmingham, Birmingham, AL

Abstract: Stroke is a major cause of locomotor disability. Nearly 800,000 people experience a stroke yearly, with 50% of ischemic stroke survivors showing lasting reductions in functional locomotor capacity. Inappropriate muscle phasing is a critical contributor to this incapacity, and occurs when a muscle is inappropriately active at a phase of the locomotor cycle during which it will interfere with limb progression, or inactive when it might otherwise contribute to limb progression. Prior work in pedaling, which provides a mechanically-constrained cyclical locomotor-like task that allows well-controlled study of phasing, has demonstrated prolonged activation in the paretic limb of vastus medialis (VM), a uniarticular knee extensor, that is the major contributor to progression of the limb during the downstroke. This prolonged activation also appears in walking, and impairs appropriate initiation of knee flexion during swing. In this pedaling study, following a control period, we presented individuals poststroke with visual feedback of activity in the VM, and a deactivation target at the earliest behaviorally-relevant angle found in the literature (seen during very high-frequency pedaling) for five 3-minute trials at 40rpm. We hypothesized that individuals would advance deactivation in both paretic and non-paretic VM, but that the shift would be reduced in the paretic limb. Using a novel method presented previously by the authors at SfN 2014, the overall relative timing of activation during each individual cycle was calculated relative to control period activation. Participants (n=12), who had experienced a stroke ≥5 months before the study, demonstrated a significantly earlier median muscle phasing during the feedback condition compared to control, for both the non-paretic and paretic limb (Wilcoxon rank-sum, p<0.005). The non-paretic limb showed advancement of 16.4±21.9° across participants, with 10/12 medians advanced. Surprisingly, the paretic shift showed advancement of 24.5±31.3°, with 11/12 medians advanced. The larger shift in the paretic limb is likely to reflect a greater difference between deactivation during the control period (in which the paretic limb shows delayed deactivation) and the final target. However, this result clearly shows that despite impaired phase of activation during typical behavior, individuals poststroke demonstrated a considerable ability to manipulate muscle phasing in the paretic limb, contrary to current theories suggesting that the poststroke nervous system has reduced capability for flexible activation/deactivation of muscle activity during movement.
Title: Dynamics inherent to bipedal locomotion constrain active control of foot placement

Authors: *S. L. BARTON*\(^1\), J. S. MATTHIS\(^1\), B. R. FAJEN\(^2\);
\(^1\)Dept. of Cognitive Sci., \(^2\)Rensselaer Polytechnic Inst., Troy, NY; \(^3\)Ctr. for Perceptual Systems, Univ. of Texas, Austin, Austin, TX

Abstract: When walking over complex terrain, humans use visual information to exploit the passive physical forces inherent to bipedal locomotion. We previously showed that control of stepping to a target foothold relies critically on visual information of that target during the end of the preceding step (Matthis & Fajen, 2013; Matthis, Barton, & Fajen, 2015). Visual information about target position presented during this "critical control phase" was sufficient for subjects to accurately step to a target, while the same information presented before or after the critical phase resulted in stepping errors. These findings reflect a control strategy based on the mechanical structure of the body, in which visual information is used to initialize a ballistic trajectory of the body for the upcoming step. Although visual information about target position is critical during the end of the preceding step, it remains unclear if humans use visual information about target footholds during a step to make adjustments to the foot's trajectory mid-flight. Such active control may be important for responding adaptively to changes in the environment that render a previously safe target foothold undesirable or unobtainable. However, mid-flight corrections during continuous walking, where stability and efficiency are largely governed by the passive dynamics initialized prior to each step, are likely to be constrained by the forces inherent in bipedal locomotion. In the present study we explored the contributions of active and passive modes of control by asking subjects to walk along a path of irregularly spaced targets projected onto the ground while their movements were recorded with motion capture. On a subset of trials, the location of one of the six targets was perturbed in either a medial/lateral or anterior/posterior direction as subjects approached. Perturbations were applied at one of four points during
subjects’ approach, three of which occurred during the step to the target, and one that occurred during the preceding step. We found that subjects’ ability to respond to the perturbation depended strongly on the point at which the perturbation occurred. When the perturbation occurred at less than half a step from the target, subjects demonstrated no corrective movements, leading to large stepping errors. As the perturbation was applied earlier in the approach, stepping error decreased systematically. However, stepping error did not return to baseline in any condition in which the target was perturbed. This suggests that the ability of a walker to assert active control of foot placement during continuous walking is constrained by the dynamics of continuous locomotion.


Poster
520. Gait and Posture: Aging, Injury, and Disease

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 520.01/U8


Support: NIH Grant NS070264

MnDRIVE Postdoctoral Fellowship

Title: Effect of cue timing and modality on gait initiation in Parkinson's disease

Authors: *C. LU1,2, S. L. AMUNDSEN HUFFMASTER1,2, P. J. TUITE2, J. M. VACHON1,2, C. D. MACKINNON1,2; 1Movement Disorders Lab., 2Neurol., Univ. of Minnesota, Minneapolis, MN

Abstract: The purpose of this study was to examine the effect of different cue timings and modalities on anticipatory postural adjustments (APAs) preceding and accompanying gait initiation in Parkinson's disease (PD) patients with and without freezing of gait (FOG). Sensory cues can markedly facilitate movement initiation in people with PD, even when they are off medication. These observations provide compelling evidence that PD patients retain the capacity to initiate movement via non-dopaminergic pathways. Currently it is unknown how the timing of the cue or the cue modality impacts gait initiation in PD patients. Twentytwo patients with PD (11 freezers) were studied in the off medication state. Step initiation was cued using instructed--delay (warning-go) timing protocols: fixed delay period (3 s), random delay period (4-12 s), and countdown (3-2-1-go, 1 s between cues) in three sensory modalities: visual, acoustic and
Subjects also performed self-initiated stepping trials. The APA variables were the timing and peak amplitude of stepping leg loading force, stance leg unloading force, and initial excursions of the net center of pressure in both the mediolateral (COPml) and anteroposterior (COPap) directions. The change in APA performance between the self-initiated and cued conditions was calculated in each individual. Changes in APAs were analyzed using a repeated measures analysis of variance with factors of group, cue timing, and cue modality. There was a significant main effect (p < 0.05) of cue timing on the timing of APA generation. Post-hoc tests showed the time to peak loading of the stepping leg (p = 0.004) and peak excursions of the COPml (p = 0.002) and COPap (p < 0.001) were significantly increased in the countdown compared with the random delay condition. There was no significant effect of cue timing on the magnitude of change in the APA. A significant main effect (p < 0.05) of cue modality was found on the amplitude of the COP excursion. Peak excursions of the COPml (p = 0.042) and COPap (p = 0.003) were significantly reduced in the tactile compared with the visual condition. Group effects were not significant for all variables. The data demonstrate that cueing protocols that incorporate predictable timing improve the timing, but not the amplitude of APAs compared to random timing in PD patients. In contrast, cue modality affects the magnitude but not the timing of APAs. Visual cues improve the amplitude of gait initiation compared to tactile cues in both freezers and non-freezers.

Abstract: External cues can improve gait initiation in people with Parkinson’s disease (PD) and help those with freezing of gait (FOG) overcome a freeze, but the effectiveness of cues is often highly variable within individuals, thus reducing the clinical utility of this intervention. This study examined how the timing of auditory cues affects the variability of the anticipatory postural adjustments (APAs) that precede and accompany gait initiation. Self-initiated and cued gait initiation trials were performed by 24 PD subjects off medication (12 with FOG). Three cue timing conditions were tested (6 trials each) using an instructed-delay (warning-“go”) paradigm: fixed delay (3s), random delay (4-12 s), and countdown (3-2-1-go, 1 s between cues). Ground reaction forces (GRFs) and center of pressure (CoP) excursions from two force plates and bilateral EMG from 10 leg muscles were recorded. Filtered forces and CoP were time normalized from APA onset to step leg toe-off. The coefficient of variation (CV) of the medial-lateral (CoPml) and anterior-posterior (CoPap) CoP and vertical GRFs (GRFv) were calculated for each condition. The mean and standard deviation of peak filtered EMG were determined bilaterally for tibialis anterior (TA) and gluteus medius (GMs) EMG in each set of trials. The effect of Cue Timing and Group was tested via repeated measures ANOVA. Results showed a significant (p<0.05) main effect of Group for the CV of CoPml. Post-hoc analysis showed the CV of CoPml was higher in the FOG group. The effect of Group for the variability of the stance leg GM EMG approached significance (p = 0.07). There was a significant effect of Cue Timing for the CV of CoPml, GRFv and variability of the peak TA EMG for the step and stance legs. Post-hoc analysis showed the CV of CoPml significantly decreased for the random and fixed delay cues relative to self-initiated, and for the fixed delay relative to the countdown condition. The CV of the GRFv for the step leg was lower in the fixed delay than the self-initiated or countdown conditions. Peak TA EMG variability increased in both legs for all cueing conditions compared to self-initiated gait. Our findings demonstrate that patients with FOG have more variability in medial-lateral gait initiation profiles compared to those without FOG. External cues improved the consistency of lateral weight shift in both groups. Cues increased the variability of TA activation compared to self-initiated gait largely due to an increase in magnitude. Our results show that cues improve the reliability of gait initiation, particularly force generation in the medial-lateral plane. A fixed delay cueing paradigm provides the most consistent gait initiation performance.

Title: The feasibility and efficacy of a standing continuous tracking task: Exploring the dosing of postural task practice in Parkinson’s disease

Authors: *S. Y. SCHAEFER*1,2, H. A. HAYES2, L. E. DIBBLE2;
1HPER, Utah State Univ., Logan, UT; 2Physical Therapy, Univ. of Utah, Salt Lake City, UT

Abstract: Although Parkinson disease (PD) impairs motor learning, the paradigms documenting such deficits have not necessarily been salient to the postural dysfunctions associated with PD, particularly when unmedicated by dopamine replacement. Saliency aside, however, excess exogenous dopamine may further impair motor learning in PD despite improving motor signs themselves. The purpose of this study was to test the feasibility and efficacy of a standing continuous tracking task (CTT) for measuring motor learning. To account for age, disease, and dopamine replacement effects, we tested two groups of individuals with PD on (n=10) or off (n=9) medication, compared to two control groups of healthy elderly (n=10) and healthy young (n=10) adults. We hypothesized that the prescribed dose of practice (12 blocks of 10 repetitions over two days) would be feasible for all participants, and that the healthy control groups (HE and HY) would learn the standing CTT faster than either PD group (On or Off med), such that the prescribed dose would be less efficacious for learning in participants with PD. The CTT required anterior-posterior postural sway to track a visual target trajectory that contained a repeating pattern; root mean square error (RMSE) was recorded as the difference between this and the participant’s trajectory. Feasibility was measured as the percentage of participants who completed the required practice dose without adverse events (e.g. fatigue/falls). Furthermore, exponential decay functions were fitted to the RMSE data across practice blocks for each participant, with the decay constant indicating the number of blocks needed to achieve an asymptote in performance (i.e. steady state). Efficacy was then determined by the percentage of participants who achieved this steady state within the prescribed practice dose. We found that the dose was feasible for 100% of all groups with no adverse events. This dose was not, however, efficacious for 100% of any group to achieve a steady state in performance. In light of our hypothesis, the HY group had more participants (90%) achieve steady states within the dose compared to the other groups (HE=70%, PD On=78%, PD Off=33%). These results suggest that our standing CTT was feasible for people with and without PD. Furthermore, our practice dose was only selectively efficacious for promoting learning, and even less so off dopamine replacement medication than on. Nevertheless, future research is needed to address the
interactions between practice dose, exogenous dopamine, and age on the treatment of Parkinsonian motor deficits through non-pharmacological, learning-based interventions.


Poster

520. Gait and Posture: Aging, Injury, and Disease

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 520.04/U11


Support: NSF Neuroengineering IGERT grant #090622

- NSF Engineering Center for Compact and Efficient Fluid Power grant #0540834
- NIH grant RO1 NS070264

Title: Effectiveness of self-triggered versus externally-triggered cueing for improving gait initiation in persons with Parkinson’s disease and freezing of gait

Authors: *M. PETRUCCI¹, C. MACKINNON², E. HSIAO-WECKSLER¹;
¹Univ. of Illinois Urbana-Champaign, Urbana, IL; ²Univ. of Minnesota, Minneapolis, MN

Abstract: Freezing of gait (FOG) is a disorder that affects approximately one half of people with Parkinson’s disease (PD) and may be related to a diminished ability to couple posture and locomotion. An example of this impairment is the absence or reduction of anticipatory postural adjustments (APAs) during gait initiation. APAs are necessary to propel the body forward and laterally towards the stance foot as the stepping foot comes off the ground. Exogenous sensory cues can significantly improve the magnitude and timing of APAs and reduce the incidence of FOG episodes. However, the utility of these cues is often compromised by the inability to determine the timing and context during which the cue should be delivered. For this reason, a self-triggered cue (e.g., the user is able to press a button to receive a go-cue) might solve this problem. However, it is unclear if a self-triggered cue can be as effective as an externally-triggered cue. In the current study, we evaluated the effectiveness of an acoustic and a mechanical assistance cue using these two cue delivery paradigms. Five participants (64± 12 yrs, H-Y 3-3.5) with FOG symptoms were tested OFF medication. Four test conditions were evaluated in both the self and externally-triggered paradigms: [Baseline] - self-initiated stepping without a cue, [Acoustic] - acoustic cue (80 dB, 1 KHz tone for 500 ms), [Assist] mechanical assistance cue provided by a powered ankle-foot orthosis (sequence of dorsiflexor and
plantarflexor torque, 330 ms and 83 ms, respectively) worn on the stepping foot, and [Acoustic-Assist] both acoustic and mechanical assistance cue provided simultaneously. Participants were asked to take several steps forward on the go-cue starting with their right foot, for all trials. All externally-triggered go-cues were presented 2.5 s after a ready-cue. Vertical ground reaction forces (vGRF) and center of pressure (COP) data were collected. Preliminary results showed that APA magnitudes were increased in the externally-triggered paradigm. Compared to Baseline, vGRF and medial lateral COP (ML-COP) magnitudes were increased in the Acoustic (64% vGRF, 37% ML-COP), Assist (50% vGRF, 52% ML-COP), and Acoustic-Assist (82% vGRF, 77% ML-COP) conditions. However, decreases in these same parameters were found in the self-triggering paradigm in the Acoustic (-22% vGRF, -8% ML-COP), Assist (-32% vGRF, -29% ML-COP), and Acoustic-Assist (-4% vGRF, -9% ML-COP) compared to Baseline. These findings suggest that self-triggering may result in decreased APAs compared to the externally-triggered paradigm. Thus, a selftriggered cue may not be as effective at restoring normative gait initiation behaviors in persons with PD and FOG.

Disclosures: M. Petrucci: None. C. MacKinnon: None. E. Hsiao-Wecksler: None.

Poster

520. Gait and Posture: Aging, Injury, and Disease

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 520.05/U12


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NIH Grant T32 HS017582

Oregon Tax Check-Off Program for Alzheimer’s Research

Title: Variability in frequent, objective postural sway measures in mild cognitive impairment

Authors: *J. M. LEACH¹, M. MANCINI², J. A. KAYE², T. L. HAYES¹, F. B. HORAK²;

Abstract: Background: There are fundamental insufficiencies in infrequent measures as they reflect one instance of performance, cannot detect changes in performance variability over time, and may mask true changes in both cognitive and motor function. Motor changes have been shown to coincide with or precede cognitive changes. Because postural control (a specific motor function) and cognitive status have been evidenced as covariates, and because both postural and cognitive performance become increasingly more variable during the initial stages of decline, the longitudinal variability in postural sway (i.e., static postural control) may serve as a sensitive marker of early cognitive decline. Frequent, objective postural sway measures may differentiate older adults with mild cognitive impairment (MCI) from older adults with no cognitive impairment (NCI).

Methods: 22 subjects (11 MCI, 11 NCI) enrolled in ongoing Oregon Center for Aging and Technology (ORCATECH) studies were recruited. A Nook tablet and Wii balance board (WBB) were integrated into ORCATECH’s current in-home technological platform to quantify postural sway daily for 30 days. Subjects stood on the WBB (positioned on the floor) and performed a 3-minute daily exercise via the tablet (mounted on the wall). A cognitive load was utilized to increase the overall difficulty of the simple standing-in-place task (i.e., quiet stance). The tablet immediately transferred center of pressure (CoP) data (acquired by the WBB) and cognitive data (acquired by the tablet) to ORCATECH’s data repository via a wireless internet connection. Objective postural sway measures were derived from the CoP data and were used to quantify postural control. Cognitive data was used to make inferences regarding the subjects’ effort exerted on the cognitive dual-task. Postural dual-task costs will be calculated and postural sway will be assessed between groups. A latent class trajectory analysis will be used to assess longitudinal trends across the 30-day monitoring period.

Results: Data analysis is currently underway. If the variability in frequent, objective postural sway measures differentiates MCI from NCI, it may be used as a sensitive predictor of cognitive status and early cognitive decline.

Conclusions: This pilot study will characterize postural sway in MCI, will determine the utility of frequent, objective postural sway measures, and will lay the foundation for longitudinal, in-home monitoring of postural sway. Future work may enable early detection of cognitive decline and yield opportunities for intervention, treatment, compensation, coping, sustained independence, and prevention of irreversible damage.


Poster

520. Gait and Posture: Aging, Injury, and Disease

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 520.06/U13

Support: Northwestern Memorial Hospital

Title: Balance control is improved in children with cerebral palsy through classical-ballet based instruction

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Abstract: Cerebral palsy (CP) is the most common developmental motor disorder in children. The prevalence of CP in children in the United States is estimated to be between 3.1 to 3.6 cases per 1000 live births. Individuals with CP demonstrate abnormal muscle tone and motor control. These deficits contribute to impaired posture and control and movement coordination that in turn compromise balance control. The main goals of rehabilitation include promoting function while decreasing the risk for skeletal deformity and muscular dysfunction later in life. Rehabilitative therapies for cerebral palsy include most commonly orthopedic surgery, botulin toxin injections and physical therapy. Physical therapy interventions consist mainly of intensive stretching and strengthening exercises and more recently, high dosage robotic training aimed at restoring locomotion, reaching ability and ankle mobility. Reviews of literature fail to show meaningful clinical improvements after robotic interventions or, at best, the improvements are comparable to those of regular physical and occupational therapies. Enhancing selective motor control is at the cornerstone of this investigation and targets the ability to produce movements without unwanted, detrimental motor activity in the moving limb or in other areas in the body. Our classical ballet based intervention for movement rehabilitation targets motivation and attention as part of the rehabilitation strategy. All procedures were approved by the local IRB and informed consent and assent were obtained. Fourteen children ages 6-15, 8 male, 4 female with Gross Motor Function Classification System Scores I-IV participated in this experiment and were randomly assigned to a control or a treatment group. The treatment group participated in a one-hour class, three times per week over four weeks. Both groups received traditional physical therapy at school during the experimental period. The Pediatric Berg Balance Test (PBBT) was performed in both groups before, after, and one month after the intervention. Paired t-tests showed statistically significant improvements in the PBBT with $p<0.05$ after the intervention and one month after the intervention for the treatment group and not for the control group. Classical Ballet’s training principles align with physical therapy practices. The participants did not view the ballet classes as therapy but more as a fun activity to share with their peers. This targeted dance class proved successful in improving balance beyond the outcomes of routine physical therapy.

Disclosures: C. Lopez-Ortiz: None. T. Egan: None. D.J. Gaebler-Spira: None.
**Title:** Feasibility and effect of task-specific fall prevention training strategies to improve postural and stepping responses in patients with cerebellar dysfunction

**Authors:** *S.-J. IM, J.-H. PARK; Korea Univ., Seoul, Korea, Republic of

**Abstract:** Cerebellar dysfunction typically induces increased body instability and poor coordination of movements, which leads to high incidence of falls and fall-related injuries. There is a lack of evidence-based recommendations for effective fall prevention trainings in cerebellar patients. The present study investigated the feasibility and effect of a specific rehabilitation strategy that aims to improve dynamic balance and postural reactions against external perturbations in patients with cerebellar disease. A total of 21 patients with degenerative cerebellar disease participated in a 12-week training program, 2 times per week for 1.5 hours per session (total 24 training sessions). The rehabilitation program consisted of intensive practice of balance and gait activities that facilitate sensorimotor processing to scale the magnitude of postural responses to compensate for external perturbations. The results showed a significant reduction in the rate of falls and fear of falling after intervention. In addition, kinematic measures indicated that the training decreased the size of the postural response (i.e., body sway) and time before returning to an equilibrium point. After intervention, therefore, the patients appear to reacquire the capability to perceive and control displacement of center of gravity when challenged with external perturbations. Taken together, the present study has provided preliminary evidence that task-specific motor rehabilitation programs to prevent falls may be beneficial to enhance more timely stepping strategies in response to gravitational changes in patients with cerebellar dysfunction.

**Disclosures:** S. Im: None. J. Park: None.
**Title:** Synergistic changes in muscle activity post-stroke during split-belt treadmill walking

**Authors:** *P. ITURRALDE\(^1\)*, D. DE KAM\(^2\), G. TORRES-OVIEDO\(^1\);
\(^1\)Univ. of Pittsburgh, Pittsburgh, PA; \(^2\)Radboud Univ. Med. Ctr., Nijmegen, Netherlands

**Abstract:** Split-belt walking in which legs move at different speeds improves gait symmetry in patients who have suffered a stroke (Reisman et al. 2013). Previous work has focused on kinematic changes during this task in post-stroke patients, but little is known about the underlying changes in muscle activity. It is important to investigate the latter to understand the actual plasticity of neural mechanisms in these patients. Thus, in this study we investigated changes in muscle coordination of 16 post-stroke subjects during split-belt walking. Subjects initially walked with both legs moving at the same speed (baseline condition), then speeds were abruptly changed so that they had to walk twice as fast with their non-paretic leg than their paretic one (adaptation condition), and then once again abruptly changed to walk in the same baseline environment (post-adaptation condition). We measured electromyographic activity in 15 muscles on each leg, and compared the steady-state adapted condition measurements vs. both the baseline and the post-adaptation conditions. Statistical testing of mean activity per gait cycle phase was performed for each subject, and we used a Bonferroni correction to account for multiple comparisons. We found consistent activity changes on muscles on both legs during specific times of the gait cycle in at least 50% of the patients. Interestingly, we observed spatial and temporal symmetricity in these changes. Increased activity on one side was matched by decreased activity on the other side (spatial symmetricity) and changes in muscle activity for each leg occurred during the same phase of the ipsi-lateral gait cycle (temporal symmetricity). Specifically, we observed increased activity of hamstrings (i.e. semimembranosus, semitendinosus, and biceps femoris) in the paretic side during its swing phase matched by decreased activity contra-laterally. We also observed decreased activity of plantarflexors (soleus, medial and lateral gastrocnemius) and dorsiflexors (peroneus and tibialis anterior) in the paretic side during its stance phase matched by increased activity contra-laterally. Non-paretic rectus femoris and hip muscles were the only muscles that showed increases in activity not matched by changes in the paretic side. Because most changes in muscle activity occurred bilaterally and not only in the sound limb, our results suggest that post-stroke patients are able to modulate their muscle activity when learning a new walking pattern on the split-belt treadmill. Therefore,
chronic post-stroke patients may still have the flexibility to change their muscle coordination to improve their walking despite of their cortical lesions.

**Disclosures:** P. Iturralde: None. D. de Kam: None. G. Torres-Oviedo: None.

**Poster**

**520. Gait and Posture: Aging, Injury, and Disease**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#: 520.09/U16**

**Topic:** D.16. Posture and Gait

**Support:** NWO-Veni-2010: 'Why stroke patients fall'

**Title:** Missing muscle synergies for balance control in paretic side after stroke

**Authors:** *D. DE KAM*¹, V. WEERDESTEYN¹, G. TORRES-OVIEDO²; ¹Radboud Univ. Med. Ctr., Nijmegen, Netherlands; ²Bioengineering, Univ. of Pittsburgh, Pittsburgh, PA

**Abstract:**

**Objective:** Postural instability is a major risk factor for falls in people after stroke. Defective muscle coordination of balance recovery responses may contribute to their greater fall risk. We aimed to identify stroke related impairments in muscle control during balance recovery.

**Methods:** Ten people after unilateral stroke (> 6 months) and 9 healthy controls were subjected to translational balance perturbations in 12 directions resulting in a feet-in-place balance correcting response. Activity of eight muscles was recorded bilaterally: erector spinae (ERSP), gluteus medius (GLUT), biceps femoris (BFEM), semitendinosus (SEMT), soleus (SOL), rectus femoris (RFEM), peroneus (PER) and tibialis anterior (TA). After averaging muscle activity over 3 time bins of 75 ms, muscle synergies were extracted for each leg separately. Averaged synergy coefficients (W) and activation coefficients (c) of healthy subjects were used as a reference. To identify differences in directional tuning of synergies between patients and controls, we compared c-values in a repeated measures ANOVA for each time bin and for each synergy with LEG (paretic, non-paretic and control) and direction (DIR) as independent variables. **Results:** While three muscle synergies (W1-W3) were consistently found in healthy controls, we found differences in these synergies and their recruitment in the paretic leg of post-stroke patients. Muscle synergies W3 (BFEM, SEMT, ERS and PER) and W2 (TA, RFEM) that responded to forward translations were either missing or had lower activity compared to controls. Namely, muscle synergy W3 was absent in paretic legs of 4 stroke patients with lower clinical motor score (Moticity Index and Fugl-Meyer). Also, the activation of W3 was missing in response to
forward translations on the non-paretic side (LEG x DIR p=0.001). Similarly, the activation of muscle synergy W2 in response to forward floor translations was lower in the paretic leg compared to controls (LEG x DIR p=0.018 in bin 1 and p=0.008 in bin 2). Finally, while synergy W1 (SOL, GLUT) was present in all stroke patients, its activation C1 was higher in patients than in controls in response to medial floor translation during later time bin 3 (LEG x DIR p<0.001).

Conclusion: Stroke related impairments in postural muscle responses include absence of the 'hamstring synergy' W3 and lower recruitment of the 'anterior synergy' W2 in response to forward floor translations, when balance is mostly challenged. These deficits may contribute to the high fall rates in stroke patients.

Disclosures: D. De Kam: None. V. Weerdesteyn: None. G. Torres-Oviedo: None.
control by experiencing flat and incline conditions in two separate sessions. Split-belt perturbations and slopes were smaller for stroke patients to ensure they would be able to complete the split-belt inclined condition. Specifically, 3:1 split-belt perturbation and 8.5 degree slopes were used for controls, whereas 2:1 and 5.0 degree slopes were used for stroke patients. Kinematic data were recorded in all groups with a motion tracking system via reflective markers placed bilaterally on the trunk and legs. We computed step length symmetry in each group, which is a measure of clinical interest known to adapt during split-belt walking (Reisman et al. 2007). We evaluated the steady state reached during split-belt walking (i.e., average of the last 40 strides of adaptation) and the after-effects (i.e., average of first 5 steps of post-adaptation) as compared to baseline walking. Split-belt walking in the inclined conditions results in the greatest adapted state (p<0.001) and after-effects (p<0.001) in unimpaired subjects. Stroke patients also reached a significantly higher steady state in the incline condition compared to the flat (p=0.03), but after-effects did not change in all subjects (p=0.29). A larger sample size might result in significant differences in after-effects across conditions. In sum, incline walking on a split-belt treadmill significantly increased the extent of adaptation in unimpaired and post-stroke patients. This increase in gait adaptation is important because not all stroke patients change their movements in flat split-belt walking (Reisman et al. 2013). Therefore, it is important to identify ways to facilitate the effect of split-belt walking across patients.


Poster

520. Gait and Posture: Aging, Injury, and Disease

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 520.11/U18


Title: Effects of strength training the hip abductor-adductor muscles on protective stepping: a pilot study

Authors: *M.-L. MILLE1,2,3, M. PAPAIOORDANIDOU1, G. FLORENT1, K. EL KOULALI1, R. C. FITZPATRICK4;
1Inst. of Movement Sciences, UMR 7287, Marseille, Cedex 9, France; 2Ufr staps, Toulon Univ., La Garde, 83957, France; 3Dpt Physical Therapy & Human Movement Sci., Northwestern Univ., Chicago, 60611, IL; 4Univ. of New South Wales, Sydney, NSW 2052, Australia
Abstract: Protective stepping is a common strategy to avoid falling. Control of lateral stability during stepping is challenging, particularly for elderly subjects. It requires an inter-limb coupling of the hip abductors-adductors (AB-AD) muscles that are known to undergo age-related strength losses. Thus, strengthening of these hip muscles is hence called for since it could improve balance recovery in older people. The aim of this preliminary study was to examine the effects of hip AB-AD muscle strengthening on protective stepping. Twelve elderly subjects in good health (≥ 60 years old) were randomly assigned to a training group (TG) or to a control group (CG). Training sessions of the AB-AD muscles were performed twice a week for 8 weeks. Before (PRE) and after (POST) the 8-week period, participants underwent a battery of tests comprising AB-AD force measurements as well as protective stepping abilities. Three perpendicular synchronized rotary motors under computer control allowed forces to be applied at the waist in the forward or lateral (left or right) directions to induce protective stepping. Subjects performed 7 trials for each of the 3 perturbation directions in a random order. For each perturbation, the number of steps, the side of the first step and the first step characteristics (onset, duration, length, width and clearance) were determined. Training improved maximal contraction force of AB by 12% (Wilcoxon p<0.046) and AD by 19% (Wilcoxon p<0.046) in the TG between PRE and POST testing whereas these maximal forces were unchanged in the CG (Wilcoxon p>0.5). The percentage of trials in which only one step was made to recover balance increased from 39% to 54% in the TG (Wilcoxon p<0.027) but remained the same in the CG (PRE: 33%; POST: 39%; Wilcoxon p=0.5). Finally, first step duration decreased in the TG (PRE: 416ms; POST: 371ms; Wilcoxon p<0.027) but did not change in the CG (PRE: 427ms; POST: 397ms; Wilcoxon p=0.142). These results indicated that improving the strength of the hip abductor-adductor muscles also improves balance recovery to postural perturbation in the elderly. Thus targeting these muscles by simple strength training could be beneficial for balance and help prevent falls.

Disclosures: M. Mille: None. M. Papaioerdanidou: None. G. Florent: None. K. El Koulali: None. R.C. Fitzpatrick: None.

Poster

520. Gait and Posture: Aging, Injury, and Disease

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 520.12/U19


Support: Howard Hughes Medical Institute
Title: Time course of nanoparticle drug delivery to prevent or reverse functional and structural changes of proprioceptors in short-term hyperglycemic rats

Authors: *V. K. HAFTEL¹, J. LAKE², R. BUTLER¹, T. JOHNSON¹, M. PRIEST¹, K. MOTON-MELANCON³, C. EADDY¹;
¹Dept of Biol., ²Psychology, Morehouse Col., Atlanta, GA; ³Biol., Spelman Col., Atlanta, GA

Abstract: Short term hyperglycemia leads to dysfunction of peripheral neurons leading to numbness, tingling, decline in H-reflexes, and difficulty walking. This study was done to determine the appropriate time course of drug application via biodegradable nanoparticles (NP) to prevent/reverse changes seen in proprioceptor function in a model of Type I diabetes. Several groups of rats were injected with streptozotocin, and blood glucose monitored to allow 2 or 3 weeks of hyperglycemia (> 250 mg/dL; dpn). Then, NPs encapsulating human recombinant insulin were injected into left triceps surae muscles. One or two weeks following NP injection, terminal experiments were performed in the following groups: 3wk dpn/then 2wk NP; 3wk dpn/1wk NP; 2wk dpn/2wk NP; 2wk dpn/1wk NP. These time points were chosen based on changes in structure and function seen by us at 3 and 6 wks of hyperglycemia in previous experiments, and the time course of drug released from the formulation of NP used (insulin release of 110-10 ng/mL over 2-14 days from 75:25 of poly-lactide-co-glycolide NPs). Intraxonal electrophysiological measurements were made in anesthetized rats, and immunohistochemistry was done to determine sciatic axon:myelin diameter ratio, dorsal root ganglion soma area, and sodium channel distribution in the peripheral nerve. Data show NP injection improves muscle spindle afferent responses to muscle stretch in a time-dependent manner, e.g., afferents from 3wk dpn rats show very high no. action potentials (N) and maximum firing rate (Mx) upon muscle stretch; 6 wk dpn afferents show very low N and Mx (previous data from this lab); NP treated afferents in 3wk dpn/1wk NP, 3wk dpn/2wk NP, 2wk dpn/2wk NP resulted in gains toward untreated control values of N and Mx. Structural changes were found in dpn rat nerves, and 3wk dpn/1wk NP and 3wk dpn/2wk NP dorsal root ganglia soma areas move closer to untreated control. Measurements are being collected from the other NP groups. Axon:myelin diameter ratios of 3 wk and 6 wk dpn nerves were larger than control, and 3wk dpn/1wk NP treated side were greater than those, suggesting an inflammatory response that requires further examination. 3wk dpn/1wk NP untreated side, and 3wk dpn/2wk NP untreated side measurements were closer to control, even slightly larger. These results require more thorough examination and reanalysis. Also, sodium channel distribution in sciatic nerves from each group are being examined immunohistochemically. Further tests will be done on additional time points of NP treatment and different methods of delivery of NP to the affected neurons in order to fine tune treatment methods for peripheral changes in short term diabetes.

Title: A novel use of commerically available technology to assess balance impairment in mild traumatic brain injury

Authors: *W. WRIGHT*¹, J. MCDEVITT¹, R. TIERNEY¹, F. J. HARAN², K. APPIAH-KUBI¹; ¹Temple Univ., Philadelphia, PA; ²Naval Exptl. Diving Unit, US Navy, Panama City, FL

Abstract: Mild traumatic brain injury (mTBI) can cause diffuse axonal injury, which can affect cognitive, and emotional, and sensorimotor processes such as balance impairment. Assessments of balance following mTBI often include the Sensory Organization Test (SOT) and the Balance Error Scoring System (BESS). The SOT has shown that visual-vestibular processing deficits may underlie post-concussion balance impairment, but this test is less sensitive to unremitting symptoms that do not spontaneously resolve within a week. The BESS is most sensitive to symptoms only in the first few days post-injury. Our current project involves demonstrating validity and reliability of a novel low-cost, portable virtual reality-based balance screening device that employs established principles of sensorimotor reweighting and visual-vestibular integration. The goal is to determine if it can replace existing tools that are either prohibitively expensive or lack reliability or sensitivity. Healthy young adults (n=27) were tested to establish healthy norms. Individuals with recent mTBI (n=8) were compared to the healthy norms. The new VR-based balance assessment system consists of a Wii balance board (WBB), a large screen television, and a custom-designed software user interface which collects and processes data. Subjects performed six upright postural tasks modeled after the Clinical Test of Sensory Organization and Balance (CTSIB) with 3 visual conditions either standing directly on the WBB or on foam placed on the WBB. The three visual conditions were Static Scene, Dark Scene, and Dynamic Scene (Roll at 60 deg/s). Subjects viewed a VR scene displayed on a 60” TV. The WBB recorded center-of-pressure (COP) at 100Hz for 30 sec, and COP velocity, RMS, and sway area were calculated. Subjects also performed the BESS and SOT. Within-group repeated-measures ANOVA showed the device reduced postural stability as sensory input reliability and availability decreased. Between-groups ANOVA reveal that individuals with mTBI have significantly worse balance scores on the new VR-device (p<0.001). Criterion validity of the new device measured using ROC curves reveal sensitivity/specificity higher than BESS and
In conclusion, this study demonstrates that our new VR-based assessment tool is a valid measure for detecting balance related changes in neurologically impaired individuals and can potentially replace much more expensive equipment. Including sensitive and specific postural control measures after brain injury may help improve identification of individuals with sub-acute symptoms which can guide rehabilitation and clinical decision-making.


Poster
520. Gait and Posture: Aging, Injury, and Disease

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 520.14/U21


Support: WLU Equipment Grant

Title: Assessing the effect of repeated sub-concussive head blows on balance control in football defensive linemen

Authors: *M. CINELLI, N. FIGUEIRA;
Dept. of Kinesiology & Physical Educ., Wilfrid Laurier Univ., Waterloo, ON, Canada

Abstract: Previous research has revealed that concussed athletes experience balance deficits that are more prominent in the anterior-posterior A/P direction as indicated by increased Center of Pressure (COP) displacement and velocity which persist even when they return to activity. This deficit may be due to potential damage to the vestibular system, primarily the impairment of the lateral vestibulospinal tract which sends signals to control ankle extensors. This may lead to increased compensatory torques about the ankle. The purpose of this study was to determine if there is a sensitive enough balance measure that is able to quantify any negative cumulative effects of sub-concussive head blows on balance control of football defensive linemen. It was hypothesised that as the balance task became more challenging, a higher order analysis would be able to demonstrate any cumulative effects of sub-concussive blows. Seven varsity defensive linemen participated in balance testing every week following their Saturday game. To assess static balance during quiet stance, ground reaction forces were collected for 45s at 100Hz using a Nintendo Wii board. Participants stood on the Wii board using a narrow Romberg stance with their hands by their side during five conditions: 1) eyes open (EO); 2) eyes closed (EC); 3) math
dual task (Math); 4) an iPad dual task (iPad); and 5) horizontal head turns for 15s followed by 30 additional seconds of static stance with eyes closed (HeadTurn). Results revealed that there was no change in COP displacement or M/L COP velocity over the course of a competitive football season. However, A/P COP velocity significantly decreased ($p<.05$) in three of the conditions (EC, Math, HeadTurns) over time indicating an improved level of balance control over the course of the season. As previously reported, AP COP velocity is a sensitive enough measure to detect balance control changes over time. However, the findings from the current study indicate that exposure to repeated sub-concussive head blows during a competitive football season did not have a negative concussion-like cumulative effect. The improvement in balance control is most likely due to the neural adaptations occurring as a result of the sport specific training over the course of the season. It is possible that the improvement in strength as a response to the physical training improved both the neural and physical aspects necessary for an improvement in overall balance control. Unfortunately, the current findings are not able to determine whether or not long-term negative effects on balance control will develop following exposure to sub-concussive head blows.

**Disclosures:** M. Cinelli: None. N. Figueira: None.

**Poster**

**520. Gait and Posture: Aging, Injury, and Disease**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 520.15/U22

**Topic:** D.16. Posture and Gait

**Support:** NIH Grant R21HD080398

**Title:** Locomotion and interhemispheric motor connectivity in mTBI

**Authors:** *L. A. KING*, B. W. FLING, C. SWANSON, J. CHESNUUT; 1Neurol., 2Orthopedics and Rehabil., Oregon Hlth. & Sci. Univ., Portland, OR

**Abstract:** Despite recent data suggesting that gait coordination may be impaired after mild traumatic brain injury (mTBI), gait is not typically assessed post-concussion. Abnormality in gait coordination could 1) indicate abnormal brain functioning and 2) impair physical function and predispose the person to additional injury. The purpose of this pilot study is to objectively measure gait coordination along with structural and functional measures of inter-hemispheric motor connectivity in subjects with chronic mTBI. Three people with chronic mTBI (23, 44, and 59 years old) performed standardized gait task. Gait coordination measures included 1) phase
coordination index (PCI), and 2) stride width obtained using inertial sensors (APDM system) and Gait Rite. Resting state functional and diffusion-weighted images were collected to assess functional and structural connectivity between the right and left supplementary motor areas (SMA). Functional and diffusion images underwent well-established preprocessing techniques measuring 1) functional connectivity strength between the right and left SMA and 2) fractional anisotropy of fibers connecting those areas. Results are presented as mean (+/- SD) and are compared to normative data as appropriate. People with mTBI had reduced gait performance with larger PCI [3.7 +/-2.5 vs. 2.5 +/-0.59] and stride width [13.6 cm +/-1.6 vs 10.0 cm] compared to normative data. Fractional anisotropy of fibers connecting the bilateral SMA was reduced in all people with mTBI compared to age-matched controls [FA = 0.55 +/-0.15 vs 0.68 +/- 0.06]. Furthermore, two people with mTBI had functional hyperconnectivity between the right and left SMA compared to age-matched controls [r = 0.72 +/-0.07 vs 0.53 +/-0.08]. The third person with mTBI had reduced integrity of connecting fiber tracts to such an extent that they showed a near complete loss of functional connectivity between the SMA (r = 0.11). Consistent with existing literature we show that people with mTBI have reduced fiber tract structural quality within the body of the corpus callosum. However, this pilot data suggests that this decreased structural connectivity is related to increased functional connectivity between the higher-order motor areas. These results support the notion of a release from GABA-ergic mediated inhibition with declining interhemispheric fiber tract structure. Further, this small group of people with chronic mTBI had impaired interlimb coordination during gait possibly related to impaired interhemispheric communication. Future work will investigate associations between interhemispheric sensorimotor connectivity and gait performance.


Poster

520. Gait and Posture: Aging, Injury, and Disease

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 520.16/U23


Support: Gratama-Stichting, 2014-08

Graduate School of Medical Sciences

 Jacobs University scholarship to SP

Title: Neural correlates of dual-tasking in young and old adults
Authors: *S. PAPEGAAIJ*, T. HORTOBÁGYI, B. GODDE, P. ERHARD, C. VOELCKER-REHAGE

Univ. of Groningen, Univ. Med. Ctr., Groningen, Netherlands; Ctr. for Human Movement Sci., Univ. of Groningen, Univ. Med. Ctr. Groningen, Groningen, Netherlands; Jacobs Univ., Bremen, Germany; Univ. Bremen, Bremen, Germany

Abstract: The ability to perform two tasks simultaneously has been shown to decrease with age. One proposed neurological mechanism explaining these findings is that the age-related increase in neural recruitment to perform a single task results in a reduced residual capacity in shared brain resources when performing a dual-task (Van Impe et al., 2011). The purpose of the study was to determine whether this structural interference indeed mediates the age-related decrease in dual-task performance. Functional magnetic resonance imaging (fMRI) was used to investigate 23 young adults (20-29 years) and 32 old adults (66-89 years) performing a calculation and balance-simulation task separately or simultaneously. The calculation task consisted of serial subtraction by seven. Standing balance was simulated by a plantar flexion force control task with the goal of keeping an avatar on the screen from swaying forward or backward. Motor and calculation performance decreased during the dual-task compared with the single tasks in both age groups. There were no significant age-related differences in dual task costs. Brain activation was more extended in old than young adults during all conditions (balance, calculation and dual-task). This increased brain activation was more prominent in the old adults with low compared with high balance performance. However, brain activation did not differ between old adults with low or high dual-task costs. Moreover, dual-task costs did not seem to be related to the magnitude of upregulation in brain activity between single and dual-tasks within shared brain regions. Under the present experimental conditions the age-related increases in brain activation were related to single task performance but not to dual-task costs. Van Impe, A., Coxon, J.P., Goble, D.J., Wenderoth, N., Swinnen, S.P., 2011. Age-related changes in brain activation underlying single- and dual-task performance: visuomotor drawing and mental arithmetic. Neuropsychologia. 49, 2400-2409.

Disclosures: S. Papegaaij: None. T. Hortobágyi: None. B. Godde: None. P. Erhard: None. C. Voelcker-Rehage: None.

Poster

520. Gait and Posture: Aging, Injury, and Disease

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 520.17/U24

Support: Office of Research and Sponsored Programs, University of Michigan-Flint

Title: Dynamic parameters of postural control during reaching tasks: which are linked to fall risks for older adults living in the community?

Authors: *M.-H. HUANG¹, K. NEWMAN¹, T. SHILLING¹, A. RIGHTER¹, K. MILLER², K. SMITH³, K. FREDRICKSON⁴;
¹Univ. of Michigan-Flint, Flint, MI; ²Beaumont Hlth. Syst., Royal Oak, MI; ³Life In Balance Physical Therapy, Nile, MI; ⁴Mary Free Bed Rehabil. Hosp., Grand Rapids, MI

Abstract: Age-related changes in postural control are major risk factors of falls, leading to fall-related injuries, increased morbidity and mortality in older adults. No consensus has been established on the measures of postural stability during movements to identify those at risks of falling. This study investigated whether center of pressure (COP) parameters during reaching to objects of different loads would differ between young, older adults without (non-fallers) and with (fallers) a history of falls. 17 young adults (25±2.1 years), 11 non-fallers (65±9.2 years), and 12 fallers (68±7.3 years) living in the community volunteered. All were able to walk >50 ft without another person’s assistance and with no history of neurologic conditions. Participants reached forward to grasp a basket placed at 30 cm in front of the feet on the floor using both hands, and returned to an upright posture while holding the basket. Participants were instructed to perform the task as fast as possible. The basket’s weight was altered in the following sequence: 2.2 Kg, 0 Kg, and 2.2 Kg. Participants performed 3 trials in each load condition while data from the 1st and 3rd trials of a condition were analyzed. Center of pressure (COP) was analyzed during 3 phases of the task: anticipatory postural adjustments (APA), reaching to the target, and returning to upright. Parameters included COP displacement during APA, as well as maximum COP forward displacement, COP peak velocity, and COP trajectory smoothness during reaching and returning. Linear Mixed Model was used to analyze postural parameters, with group as between subject factor, trial as within subject factor, and Tukey’s LSD for post-hoc comparisons. Significance level was p<0.05. COP parameters were comparable across trials. APA amplitudes and durations were significantly larger in fallers compared to non-fallers and young adults (p<0.01). During reaching and returning, fallers had significantly smaller COP forward displacement than non-fallers (p<0.05) and young adults (p<0.01). In contrast, all groups achieved maximum COP forward displacement at comparable timings and had similar COP peak velocities. Moreover, COP trajectory smoothness was significantly reduced in fallers compared to non-fallers and young adults (p<0.01). Older adults retain the ability to modulate APA according to the weight of reaching targets. However, fallers achieved this by altering APA amplitudes and durations. The control of COP trajectories during movement execution is compromised with aging and a history of falls. COP variables may be sensitive and precise parameters in identifying older adults with fall risks.

Poster

520. Gait and Posture: Aging, Injury, and Disease

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 520.18/U25


Title: Does aging with a cortical lesion increase fall-risk: Examining effect of age versus stroke on intensity modulation of reactive balance responses from slip-like perturbations

Authors: *P. PATEL, T. BHATT;
The Univ. of Illinois At Chicago, Chicago, IL

Abstract: Background: We examined the effect of aging with cortical lesions on postural stability and compensatory stepping response to increasing intensity of sudden slip-like stance perturbations among older stroke survivors. We hypothesized that although older stroke survivors and healthy controls would demonstrate greater postural instability at higher perturbation intensities, older healthy adults and stroke survivors as compared with young adults would show impaired modulation of reactive balance response for recovery from perturbation. Further, at higher perturbation intensities, older stroke survivors would demonstrate least postural stability at touchdown resulting from an inefficient compensatory step and reduced ability to control trunk extension. Methods: Ten chronic older stroke survivors (56 ± 6.48 yrs), healthy age-similar controls (AS, 57.9 ± 6.4yrs), and young adults (YA, 24.5 ± 3.8 yrs) were exposed to 3 levels of forward perturbations (accelerations 7.5, 12, and 16.75 m/s² for levels I, II, and III respectively) in a random order. Stability at liftoff (LO) and touchdown (TD) was measured as the shortest distance of instantaneous center of mass (COM) state [position and velocity relative to base of support (BOS)] from a theoretical threshold for backward balance loss. Compensatory step length and trunk angle at TD were recorded. Results: All three groups showed greater instability at LO with increasing perturbation intensity (main effect of intensity, p \leq .05). At TD, while the YA had a linear improvement in stability with increasing perturbation intensities, such a trend was absent in AS and stroke groups (intensity x group interaction, p \leq .05). Thus, between group differences in stability at TD were observed at level III (YA>AS>stroke group, p \leq .05). At level III, while the YA group increased step length and reduced trunk extension to maintain COM more anterior to BOS, the stroke group was unable to do so. Although the AS group was unable to modulate step length, they reduced trunk extension at TD to achieve greater stability at level III as compared with stroke group. Conclusion: The findings reflect impairments in reactive balance control among older stroke survivors compared with their healthy counter parts, particularly at higher perturbation intensities. While aging had
an effect on intensity modulation of postural stability, the effect of aging superimposed with a cortical lesion elevates the balance deficits, explaining the increased fall risk in chronic ambulatory stroke survivors. Balance assessment and intervention in this population should incorporate more challenging environmental threats to unmask and retrain reactive balance.

Disclosures: P. Patel: None. T. Bhatt: None.

Poster

520. Gait and Posture: Aging, Injury, and Disease

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 520.19/U26


Support: NIH Grant P30 AG024827

Title: Thinking and walking while thinking outcomes of exercise interventions in older adults with age-related psychomotor slowing

Authors: K. A. LOWRY1, W. J. FARRINGTON1, *J. M. VAN SWERINGEN2; 1Des Moines Univ., Des Moines, IA; 2Univ. Pittsburgh, Pittsburgh, PA

Abstract: Purpose: Walking problems are common and often cooccur in older adults with executive cognitive deficits. We compared aerobic and motor skill, task-oriented walking exercise on cognitive, and integrated walking while thinking outcomes. Participants: Fourteen adults>65 years old with slow gait (≤1.0m/s, but >0.60m/s) and psychomotor slowing (Digit Symbol Substitution Test <age-adjusted norm), randomized to motor skill or aerobic gait exercise interventions, 12 weeks, 2 times/week. Materials/Methods: All measures completed pre and post intervention. Computerized cognitive tasks: 1) Flanker, determine direction of middle arrow in a row, interference suppression, 2) Update, add 2 for a blue circle, or subtract 1 for a red square, cognitive flexibility, 3) GoNoGo, inhibitory control, 4) 2Back, determine if number matches number two back, working memory. The same cognitive tasks were completed while walking around a track viewing the tests displayed on easels. A triaxial accelerometer secured at the waist recorded anteroposterior (AP) and vertical (V) trunk accelerations to determine smoothness, a measure of the motor control of walking. Accuracy scores were determined pre and post intervention for the computerized cognitive tasks and during the walking while thinking tasks. Results: Descriptive prepost changes in cognitive accuracy and smoothness during the walking while thinking tasks were determined; lower/negative numbers indicate greater improvement. Mean prepost changes in accuracy scores for the cognitive tasks were: 1) motor skill group,
Flanker = .05, Update = .04, GoNoGo = .01, 2back = .06; 2) aerobic group, Flanker = .02, Update = .06, GoNoGo = .04, 2back = .04. Mean pre-post changes in cognitive accuracy during the walking while thinking tasks were: 1) motor skill, Flanker = .01, Update = .05, GoNoGo = no change, 2back = .03; 2) aerobic, Flanker = no change, Update = .02, GoNoGo = no change, 2back = .02. Mean pre-post changes in AP and V smoothness during walking while thinking were: 1) motor skill, Flanker AP = .14, V = .004, Update AP = .16, V = .24, GoNoGo AP = .38, V = .50, nback AP = .50, V = .51; 2) aerobic, Flanker AP = .12, V = .06, Update AP = .26, V = .26, GoNoGo AP = .12, V = .27, nback AP = .05, V = .35. Conclusions: While the aerobic group had more consistent improvement in cognitive task accuracy, the motor skill group tended to exhibit greater and more consistent improvement of walking smoothness and cognitive accuracy during walking while thinking. Based on the preliminary data motor skill training may reduce cognitive resource burden during walking by improvements in motor control and automaticity of gait in older adults.


Poster

520. Gait and Posture: Aging, Injury, and Disease

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 520.20/U27


Support: NSF 0957920

Title: Interaction effect of Vision and Unstable base of support with Age on postural sway frequency

Authors: *E. PARK¹, G. SCHÖNER², D. REISMAN³; ¹Sch. of Applied Physiol., Georgia Inst. of Technol., Atlanta, GA; ²Ruhr-Universität, Institut für Neuroinformatik, Germany; ³Univ. of Delaware, Newark, DE

Abstract: The postural control process requires a complex integration of visual, somatosensory and vestibular sensory information about the orientation and position of body segments relative to each other, and to the surrounding environment. Age effects on postural control get attention in motor control research because the ability to maintain upright standing posture declines with aging. However, most of the findings from previous studies to investigate age effects on postural control do not address whether the effect of age comes from the control of certain joints (e.g. ankle joint), or from a different coordination strategy to stabilize posture. In addition to that, our
understanding of the interactions between factors affect postural control is still not sufficient to address the decreased postural control observed with aging. Young (YA) and older adults (OA) groups were asked stand on normal floor (NO) and soft foam base of support (FO) with eyes-opened (EO) and eyes-closed (EC) for 3-min. We applied the UCM analysis in frequency space to investigate the effect of age, vision and proprioception on coordination between joints. We distinguished the COM sway into two different frequency bands (e.g., 0–0.1Hz, and 0.1–0.5Hz) to understand whether one frequency band has more effect on postural control with age, vision and proprioceptive control. Results showed that removing visual information does not affect all frequency bands (0–0.5Hz) when both age groups stand on normal floor, however, if subjects stand on foam condition, both variance components of the UCM analysis increase in the PSF (0.1–0.5Hz) frequency band. When subjects stand on an unstable base of support, the system uses more flexible joint coordination (i.e., increase of Vucm) in all frequency bands (0–0.5Hz), accompanied with the increase of the error component of variability (Vort). In conclusion, regardless of any age group, perturbation of proprioceptive information affects COM sway in all important frequency band (0–0.5Hz), but removing visual information effect on the PSF (0.1–0.5Hz) of COM sway, only when subject stand on unstable base of support.

Disclosures: E. Park: None. G. Schöner: None. D. Reisman: None.

Poster

520. Gait and Posture: Aging, Injury, and Disease

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 520.21/U28


Support: Grant-in-Aid for Young Scientists (B) #25870164

Science Research Grant from the Ministry of Health, Labour, and Welfare, Japan H21-23-Choju-G-006

Title: Age-related deterioration in multi-joint coordination increases center of mass acceleration during quiet standing in humans

Authors: *S. SASAGAWA¹, H. OBATA², N. KAWASHIMA³, T. OGATA³, K. NAKAZAWA²;
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**Abstract:** Elderly individuals usually exhibit greater postural sway during quiet standing. In particular, the amplitude of the translational acceleration of the body’s center of mass (CoM) has been demonstrated to be much greater in the elderly than in young adults. This study investigated a kinematic mechanism by which CoM acceleration during quiet standing increases with advancing age, given a recent observation that the human quiet standing is a multi-joint motor task. Seven healthy young and eight healthy elderly participants were required to stand quietly on a dual-plate force platform. Angular motions of the ankle, knee, and hip joints were measured in the sagittal plane with an optical motion capture system. As expected, the root mean square (RMS) of the translational CoM acceleration was significantly greater in the elderly than in the young; however, there were no significant differences in the RMSs of the individual joint angular accelerations between the two age groups. The combined variance of the joint angular accelerations (in the angular acceleration space) along a direction in which the variance affects the translational CoM acceleration was significantly greater in the elderly group. These results suggest that changes in multi-joint coordination, rather than increased variability of individual joint motions, are a major cause of the impaired postural steadiness common in elderly individuals.

**Disclosures:** S. Sasagawa: None. H. Obata: None. N. Kawashima: None. T. Ogata: None. K. Nakazawa: None.

**Poster**

520. Gait and Posture: Aging, Injury, and Disease

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 520.22/U29

**Topic:** D.16. Posture and Gait

**Title:** Deficits in medio-lateral balance control, leg strength and reaction time contribute to the increased risk of falling in persons with multiple sclerosis

**Authors:** *S. MORRISON*¹, J. J. SOSNOFF², C. RYNDERS³;
¹Physical Therapy, Old Dominion Univ., Norfolk, VA; ²Kinesiology and Community Hlth., Univ. of Illinois, Urbana-Champaign, IL; ³Sch. of Med., Univ. of Colorado, Aurora, CO

**Abstract:** A major health risk faced by persons with Multiple Sclerosis (MS) is the heightened risk of suffering a fall. The reason for this increased risk can often be traced back to a general decline in neurophysiological mechanisms underlying optimal balance control and/or strength. The aim of the current study was to assess differences in falls risk, strength, reaction time (RT), and postural control between fallers with and without MS (MS: n=20, age 43-74 years;
comparison group: n=16, age 60-79 years). Falls risk was measured using the Physiological Profile Assessment (PPA). The PPA is a validated tool which includes tests of vision, sensation, posture, and leg strength. Values from each test are combined to provide an overall risk score with higher scores denoting greater falls risk. Balance was assessed using a force platform under the following conditions: 1) eyes open/firm surface, 2) eyes closed/firm surface, 3) eyes open/foam surface, and 4) eyes closed/foam surface. Simple reaction time was assessed for the hand and foot (15 trials). Results demonstrated that, in comparison to healthy older adults classified as fallers, those persons with MS had a significantly higher falls risk, slower reaction times (with the hand) and weaker lower limb musculature. For the balance measures, persons with MS exhibited greater overall postural sway compared to the comparison group. Interestingly, for the MS group their increased sway was characterized by motion in the medio-lateral (ML) direction whereas the comparison group tended to sway more in the anterior-posterior (AP) direction. Overall, the results confirm that persons with MS are often at a heightened risk of falling, due to the multitude of neuromuscular changes brought about by this disease process. However, the increased ML sway seen for the MS group could reflect a decreased ability to control motion in this direction in comparison to being able to control AP motion. Targeted interventions aimed at improving ML sway in MS may reduce the number of falls in this population.

Disclosures: S. Morrison: None. J.J. Sosnow: None. C. Rynders: None.

Poster

520. Gait and Posture: Aging, Injury, and Disease

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 520.23/U30


Support: 1T32HD071866-01

Title: Effect of assistive force applied to the center of mass during walking on peak plantarflexor kinetics of older adults and an individual with poststroke hemiparesis

Authors: *C. HURT, D. BROWN;
Univ. of Alabama At Birmingham, Birmingham, AL

Abstract: BACKGROUND/PURPOSE: Older individuals and individuals poststroke walk slowly, in part due to decreased plantarflexor power production. Assistive forces applied directly to the lower limb are shown to increase walking speed; however, these forces only affect the
swing phase of gait. Using robotic technology, we developed a paradigm that applies assistive forces to an individual’s center of mass, thus allowing undisturbed control of the lower limb throughout the gait cycle. The purpose of this study was to quantify peak plantarflexor power absorption and production while older individuals and an individual with poststroke hemiparesis walked at a comfortable speed while experiencing assistive forces. We hypothesized that assistive forces would result in decreased power production for older adults, and further, that experiencing assistive forces while walking would reduce plantarflexor power asymmetry for the individual poststroke.

METHODS: To date, we have analyzed five older individuals (62.4 years ± 9.9) and one individual poststroke (24 years). The robotic system, coupled with a split-belt instrumented treadmill, allows individuals to self-select walking speeds. Assistive forces of 30%, 60%, 90%, and 120% of the average force generation required to walk at a given speed were provided by altering the amount of average forward-directed force required by the robotic system to move at a particular speed. Individuals maintained their comfortable speed across conditions using visual feedback, and motion capture and kinetic data were collected.

RESULTS: We observed a linear trend in fore-aft ground reaction forces, indicating decreased propulsive and increased braking forces with higher levels of assistive force, but no change in power generation. We observed increased power absorption during the first half of the stance phase with increased assistive forces in 4/5 older adults. For the individual poststroke, we noted a decrease in propulsive force generation with 30% and 60% assistive force, followed by an increase at the higher levels. There was also an increasing trend in power absorption of the nonparetic limb.

DISCUSSION/CONCLUSION: For older adults, we observed increased power absorption with the addition of assistive forces, which presumably acts to slow gait speed. This may explain the lack of change in power production even though assistance was provided. For the individual poststroke, some moderate level of assistance allowed for more similar levels of power production between limbs; however, the increased power absorption of the nonparetic limb may have contributed the increase in power generation at the higher levels of assistance.

Disclosures: C. Hurt: B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; NIH. D. Brown: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); HDT Robotics.

Support: Gift

Title: Atypical muscle coordination present in hemiparetic walking as an explanation for impairment

Authors: *W. BOEHM*, K. G. GRUBEN;
1Univ. of Wisconsin Madison, Madison, WI; 2Kinesiology, Biomed. Engineering, Mechanical Engin., Univ. of Wisconsin, Madison, WI

Abstract: Following a stroke, humans with hemiparesis often present with impaired walking. Currently published perspectives of how stroke disrupts walking indicate rehabilitation methods should focus on removing kinematic deviations to restore walking, however, therapies centered on this objective have failed to produce the expected improvements for patients. Our lab has shown the plausibility of an alternative explanation for the observed behavior deviations that accounts for both the occurrence of the behaviors as well as the failure of state-of-the-art therapeutic approaches to remove them. Leg muscle coordination deviation has been observed in hemiparetic individuals during a non-balancing task. That deviation, if present during walking, would necessitate compensatory strategies such as the behaviors observed in hemiparetic gait. It was hypothesized that muscle coordination deviation is indeed present during hemiparetic walking. The ground reaction force on the foot (F), which reflects the coordination of muscles crossing the joints of the leg, was measured in stroke patients walking on a custom force treadmill. Deviation in F compared to the participant’s non-paretic limb was observed and had consistent character across all eight study participants. These findings suggest that the limb coordination used in hemiparetic walking is altered in a specific manner. Appropriate F is needed to prevent falling down or over, so the atypical F observed is likely to interfere with balancing and could elicit compensatory behaviors. Common therapeutic interventions, such as body weight supported treadmill training, provide balance support while attending to kinematic patterns. The limited success of these therapies, then, may be due to 1) not addressing learning coordination to produce appropriate F and 2) acute kinematic improvements only occurring due to external support reducing the need for compensatory behaviors and not motor learning. These findings suggest that future therapy should be developed to focus on correcting an underlying coordination mechanism rather than only addressing kinematic deviations.

Disclosures: W. Boehm: None. K.G. Gruben: None.

Poster

520. Gait and Posture: Aging, Injury, and Disease

Location: Hall A
Title: The influence of sensorimotor deficits on unexpected lateral perturbations after stroke

Authors: *V. L. GRAY, C.-L. YANG, S. MCCOMBE WALLER, M. W. ROGERS; Physical Therapy & Rehabil. Sci., Univ. of Maryland Baltimore, Baltimore, MD

Abstract: Protective stepping is commonly used as a strategy to recover and stabilize the body when one’s balance is disturbed. Increasing the base of support, with a lateral step, is the most effective means, however, crossover and medial steps are often used by people with poor balance. After a stroke, balance recovery is difficult, many falls occur as weight is transferred laterally, with an equal number of falls reported during unexpected disturbances as there are during voluntary movements. The recovery of one’s balance is impacted by the residual sensorimotor deficits, such as sensory impairments, paresis and impaired coordination. Little is known about the factors that influence step behavior when perturbed unexpectedly laterally. The purpose was to determine whether cutaneous sensation or isokinetic peak torque of the ankle (plantarflexors (PF) and dorsiflexors (DF)) and hip (abductors (ABD) and adductors (ADD)) has an impact on the step type in response to an unexpected lateral waist-pull. Fourteen community dwelling individuals (> 6 months post-stroke) were tested using a lateral waist-pull system at 3 perturbation magnitudes. Step type was recorded during 18 randomly ordered trials (3 magnitudes x 2 sides x 3 trials). Participants were instructed to react naturally and prevent themselves from falling. The peak isokinetic torque of hip ADD/ABD and ankle PF/DF was measured with a dynamometer. The paretic limb torque was calculated as a percentage of non-paretic torque. Primary outcomes were step type, hip ABD/ADD torque, ankle PF/DF torque and cutaneous sensation. Cutaneous sensation was assessed on the plantar aspect of the foot with monofilaments. ANOVA was used to examine the differences in primary outcomes by step type. Three step types were used, crossover, medial and lateral step. The perturbation magnitude did not influence the step type. When participants were pulled towards the paretic side, a medial step was commonly used by those individuals with greater deficits in sensation and torque of the paretic ankle PF and hip ABD. A lateral step with the paretic leg was used by those with minor deficits in sensation and paretic hip ABD and ADD torque. When pulled to the non-paretic side, neither sensation nor paretic torque deficits were significantly related to the step type. The results suggested, a medial step is more frequently used to recover lateral balance when pulled towards the paretic side for persons with weaker hip abductor torque and greater sensory impairments.
This may indicate a compensatory strategy that is biomechanically less effective, however, provides a quicker response to stabilize the body.

**Disclosures:** V.L. Gray: None. C. Yang: None. S. McCombe Waller: None. M.W. Rogers: None.

**Poster**

520. Gait and Posture: Aging, Injury, and Disease

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program/#/Poster#:** 520.26/U33

**Topic:** D.16. Posture and Gait

**Support:** National MS Society (RG 4914A1/2; PI: JH)

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Department of Veteran's Affairs (#I01BX007080; PI: DP)

Department of Veteran's Affairs (E1075-R; PI: FH)

NIH (R01 AG006457 29 PI: FH)

Medical Research Foundation of Oregon (PI: DP)

**Title:** Compensatory stepping in people with multiple sclerosis

**Authors:** *D. S. PETERSON*¹², J. HUISINGA³, F. HORAK¹²;

¹Oregon Hlth. & Sci. Univ., Portland, OR; ²Veterans Affairs Portland Hlth. Care Syst., Portland, OR; ³Landon Ctr. on Aging, Univ. of Kansas Med. Ctr., Kansas City, KS

**Abstract:** Our objective was to characterize compensatory stepping in people with multiple sclerosis (MS) in order to identify possible targets for stepping rehabilitation. Fifty four people with MS and 21 age-matched healthy adults underwent forward and backward support surface translations. Slow- “in-place” perturbations (4cm in amplitude, 15cm/s in velocity) and faster- “stepping-response” perturbations (15cm in amplitude, 56cm/s in velocity) were carried out. Body kinematics and shank electromyography were captured. Persons with MS also completed clinical outcomes including the European Database for Multiple Sclerosis Disability Score (EDMUS-DSS) and 25ft timed walk. People with MS exhibited more center of mass movement after perturbations and more pronounced deficits in compensatory stepping characteristics than healthy adults (e.g. step latency, number of steps, anticipatory postural responses). Stepping
deficits in MS were observed primarily during backward stepping and were correlated to clinical outcomes (EDMUS-DSS; 25ft walk time). Both healthy subjects and persons with MS exhibited slower muscle onset time after fast translations compared to slow translations. Compensatory steps are altered in people with MS, however these deficits are more pronounced during backward stepping than forward stepping which suggests backward stepping may be more effective at identifying stepping dysfunction in MS. Step latency and anticipatory postural responses are particularly altered during backward stepping in people with MS and may be appropriate targets for neurorehabilitation.

Disclosures: D.S. Peterson: None. J. Huisinga: None. F. Horak: None.

Poster

520. Gait and Posture: Aging, Injury, and Disease

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 520.27/U34


Title: Preferred sense for static balance in people with diabetes and sensation loss

Authors: R. WILKINS1, M. MARTIN1, *S. D. MOTTS2;
1Physical Therapy, Arkansas State Univ., Jonesboro, AR; 2Physical Therapy, Arkansas State Univ., State University, AR

Abstract: Diabetes can cause neurologic problems such as peripheral neuropathy. People with diabetes can have decreased foot sensation which may lead to balance impairments. Some suggest that people with sensation loss rely more on their visual system than somatosensation for balance. However, diabetes also affects the small blood vessels of the eye (retinopathy), thus vision could be declining along with foot sensation. We hypothesize that people with diabetes and sensation loss continue to rely on somatosensation for balance. This study was approved by the IRB at Arkansas State University. After obtaining consent, we tested balance and sensation in subjects who had diabetes. Subjects were tested for balance on the NeuroCom using the sensory organization test. This test consisted of six different activities that challenged somatosensation, vision, and vestibular senses. This tool determines which sense subjects rely most upon to maintain balance. Subjects were also tested for sensation using Semmes-Weinstein monofilaments on the plantar surface of both feet. Eleven persons with diabetes were tested. All subjects had diminished light touch sensation. Normal sensation is measured by the 3.61 monofilament, which requires 0.4 grams of force to bend. Two subjects had a lack of protective sensation measured by the 5.07 monofilament. The 5.07 monofilament requires 10 grams of
force to bend. The grand mean of monofilament measurements was 4.62. For balance, the sensory organization test was completed using the NeuroCom. The average equilibrium composite score was 78.78 with a range of 37-93. We hypothesized that subjects with diabetes and sensation loss would continue to rely on somatosensation for balance. We found that the majority of our subjects (7 out of 11) relied on somatosensation for balance. There is no correlation between sensation loss and sense preference for balance (Spearman’s rho correlation coefficient = -0.060). Understanding the preferred sense used for balance will allow clinicians to develop better treatment plans to help patients with diabetes deal with their neuropathy and balance deficits. One of the limitations of this study was lack of subject participation. For higher level evidence future studies need to be completed with a larger group of subjects. Another limitation to our study is that we did not know which sensation the subjects relied on before they lost sensation. Knowing previous preference of sense (somatosensation, vision, or vestibular) for balance would enable the identification of a change in sense preference as somatosensation is lost.

Disclosures: R. Wilkins: None. M. Martin: None. S.D. Motts: None.

Poster

521. Reaching and Motor Learning

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 521.01/U35

Topic: D.17. Voluntary Movements

Support: American Heart Association 0330411Z

NIH R24 HD39627
NIH 5 RO1 NS35673
NIH F32HD08658
Whitaker RG010157
NSF BES0238442
NIH R01HD053727

Title: Determination of the optimal error-augmented feedback schedules for enhancing acquisition of novel motor skills
Authors: *P. N. PARMAR*, J. L. PATTON

1Sensory Motor Performance Program, Rehabil. Inst. of Chicago, Chicago, IL; 2Bioengineering, Univ. of Illinois at Chicago, Chicago, IL

Abstract: Error-based learning mechanisms are believed to play a key role when acquiring novel motor skills and adapting to perturbations. Recently, Patton et al. (2013) demonstrated how visuomotor learning in human might be enhanced by artificially augmenting the size of perceived mistakes (Error-Augmentation or EA) in point-to-point reaching. They observed nonlinear influence of different EA gains, with faster learning rates for only the intermediate values (gain 2) and higher post-training performances for lower values (gain 1). What has not been explored is the variations in outcome that might be accomplished by allowing the EA gain to change across trials. Here, we demonstrate an optimal control modeling approach to determine the optimal EA gain schedule that best enhances visuomotor learning. We used previously acquired experimental data (Patton et al. 2013) to model a phenomenological process of visuomotor learning using Gaussian process regression. We then used Pontryagin’s minimum principle to achieve the optimal EA gain schedules that yield the fastest learning and the highest performance. Our results reveal that EA gain should be nearly doubled (×1.92) throughout training for the fastest learning. However if the highest post-training performance is desired along with the fastest learning, the optimal feedback gain schedule should gradually vary from 1.92 to 1 across training. This study explores a novel approach to optimize skill acquisition for learning to operate machines such as robotic tools, and prosthetics/assistive technologies. It also sheds light on relationships of tradeoffs between speed and final error in motor learning.

Disclosures: P.N. Parmar: None. J.L. Patton: None.

Poster

521. Reaching and Motor Learning

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 521.02/U36

Topic: D.17. Voluntary Movements

Support: MSU-Sparrow Center for Innovation and Research

Title: Introducing variability to elicit changes in bimanual coordination

Authors: *R. RANGANATHAN;
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Abstract: The ability to coordinate multiple degrees of freedom is critical to successfully perform everyday motor tasks. This coordination is often disrupted in neurological conditions such as stroke and the goal of rehabilitation is primarily to alter these coordination patterns to improve both functional outcomes and promote biological recovery. Here we look whether introducing variability during practice can successfully change the coordination patterns used to perform a motor task. We recruited 16 college-aged individuals with no history of neurological impairment. The goal of the participants was to make reaching movements with a cursor toward three targets shown on a screen (placed at 40 degrees, 90 degrees and 135 degrees with respect to the horizontal) as fast and accurately as possible. The cursor position was computed as the average location of the individual hands, which meant that the task could be achieved using different contributions of the left and right hands. If participants reached the target within 500 ms, the target turned yellow and they received a pleasant tone. Participants initially completed a baseline block consisting of 90 trials (Baseline 1). After the baseline block, we had a rotation block where we gradually introduced a visuomotor rotation on the right hand in one of two ways -- in the first group of participants, the rotation was always constant at +60°. In the second group of participants, the rotation had the same mean angle of +60° but on any given trial it was variable and picked randomly from a uniform distribution ranging from +30° and +90°. Participants adapted to this rotation for 4 blocks (360 trials) before being gradually brought back to the baseline condition (Baseline 2) again. Results showed that even though we manipulated properties of only one hand, participants adapted to both the constant and variable visuomotor rotations with curved trajectories in both hands. These changes tended to maintain symmetrical motions of the two hands. Moreover, when comparing Baseline 1 to Baseline 2, we observed changes in the contribution in the involvement of the left and right hands in the first few trials, but these differences tended to wash out with more trials. These results suggest that introducing variability resulted in reorganization of bimanual coordination, although the tendency for making symmetric movements persisted. These have implications for stroke rehabilitation where one side of the body is affected.

Disclosures: R. Ranganathan: None.

Poster

521. Reaching and Motor Learning

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 521.03/U37

Topic: D.17. Voluntary Movements

Support: NIH R01 Grant NS053606
Title: Inverse identification of motor deficits using movement distributions and mixtures of expert models

Authors: *J. R. LANCASTER¹, F. C. HUANG², J. L. PATTON¹;¹Univ. of Illinois At Chicago, Chicago, IL; ²Rehab. Inst. of Chicago, Chicago, IL

Abstract: Motor deficits resulting from stroke and other neurological disorders significantly decrease quality of life and produce a wide variety of unusual motor behaviors in affected patients. Although the clinical literature contains many terms used to describe stereotypical motor behaviors seen in this patient population, characterizations of motor impairments have generally been confined to coarse, ordinal measures of general function such as the Fugl-Meyer Assessment of Motor Recovery after Stroke. Our goal in this work is to determine if idealized models of motor deficits can be identified by observing subjects’ movement tendencies. In order to access these tendencies, we have developed a novel experimental paradigm called “free exploration.” In this protocol, subjects were instructed to grip a robotic end effector and freely explore the workspace of the robot, varying their movements through many different positions and speeds while trying to avoid repetitive motions. We used healthy control data from one of these experiments to drive a simulation of a planar two-link arm model similar to that used in Shadmehr and Mussa-Ivaldi, 1994, which was used to produce movement distributions. We also developed three idealized models of pathology (weakness, rigidity and spasticity) governed by a total of ten “pathoparameters,” which we varied in our simulation to produce “impaired” arm models. By varying each pathoparameter from zero (healthy) to one (maximally impaired), we constructed a “library” of impairment distributions that we could search in order to identify the pathoparameters underlying an unidentified distribution. We discovered using only single-impairment library elements did not give sufficient information to identify models containing combinations of impairments. In addition, as the severity of an impairment increased, more library elements were needed to provide the desired resolution. By implementing these changes, we were able to successfully identify the pathoparameters corresponding to unidentified movement distributions. This is a proof-of-concept for quantitative identification of motor impairments using measurements of movement tendencies. The framework developed here can be used with any computational model of motor impairment and can be generalized to three-dimensional space. We hope that this work will allow better characterization of motor impairments in patients suffering from neurological disorders and that this will, in turn, lead to better customization of treatments.

Disclosures: J.R. Lancaster: None. F.C. Huang: None. J.L. Patton: None.

Poster

521. Reaching and Motor Learning

Location: Hall A
**Title:** Customized forces using distributions of error improve learning a visual-motor transformation

**Authors:** *M. Fisher*\(^1,2\), F. C. Huang\(^1,3\), V. Klamroth-Marganska\(^4\), R. Riener\(^4,5\), J. Patton\(^1,2\);
\(^1\)Rehabil. Inst. of Chicago, Chicago, IL; \(^2\)Univ. of Illinois at Chicago, Chicago, IL;
\(^3\)Northwestern Univ., Chicago, IL; \(^4\)ETH Zürich, Zürich, Switzerland; \(^5\)Univ. of Zürich, Zürich, Switzerland

**Abstract:** Error feedback is critical for supporting motor adaptation in rehabilitation, sports, piloting, and skilled manual tasks. Error augmentation interventions, in which participants’ errors are amplified with either visual or haptic feedback during training has shown success over repetitive practice. Here we show that the statistical tendencies arising from free movement exploration can improve error augmentation with customized training forces that vary across the trajectory. We hypothesized that with error-augmentation customized to individual distributions of reaching errors, or error fields, participants will adapt faster to learning a visual-motor distortion and have greater improvement than participants receiving standard error-augmentation and participants repetitively practicing the task. We tested twenty-one participants using a robotic exoskeleton device restricted to two degrees of freedom. We found that the time constant of decay (measured by number of trials) was significantly lower for the EF Group (12±6 trials) than the EA and Control Groups (32±16 and 36±15 respectively). The change in error was greatest for participants receiving customized forces. These promising results support the need for customization to target subject specific errors.

**Disclosures:** M. Fisher: None. F.C. Huang: None. V. Klamroth-Marganska: None. R. Riener: None. J. Patton: None.
Title: Analysis of simple motor performance and complex procedural skill in simulated surgery tasks

Authors: *F. C. HUANG¹, D. RUTHERFORD², R. RAY², F. A. MUSSA-IVALDI¹, C. PUGH²;
¹Sensory Motor Performance Prog, Rehabil Inst. of Chicago, Chicago, IL; ²Dept. of Surgery, Univ. of Wisconsin Sch. of Med. and Publ. Hlth., Madison, WI

Abstract: Skill in surgery relies on perceptual and motor abilities, but also requires the ability to perform complex sequences and cope with unexpected events. Our goal in this study was to examine how metrics of simple motor tasks can be used to predict performance in complex procedural tasks. Our unique study design included testing with virtual environment stations and clinical scenario simulation (physical apparatuses) stations, allowing for measurement of several types of skills. We devised an interactive virtual environment using haptic devices and video displays, in which participants could perform multiple testing modules, including movements within visual distortion, force perturbation, as well as a simulated tissue puncture task. Our experimental apparatus also featured physical stations that simulated common clinical procedures for a general surgery resident. These included a laparoscopic ventral hernia (LVH) repair, bowel anastomosis, central venous catheterization, and bladder catheterization. We considered several metrics that reflected basic sensorimotor capabilities, for example, overall movement accuracy, task completion time, and maximum speed. We also considered high-level descriptions of behavior, including coping with changes in task conditions and decision-making.

Our preliminary analysis has focused on performance in the virtual environments tests. We found that surgery residents (n=47) were sensitive to changes in specific task conditions, including the starting location of movement, heading angle of movement, the degree of visual distortion, and coping with changes in the force threshold of the puncture task. Interestingly, the changes in movement heading and starting location were significant factors across different tasks. The identification of these key metrics of motor performance will allow for analysis of the relationships between skills in simple motor tasks versus complex procedural tasks. Future work will also consider how such metrics can be used to predict clinical performance in terms of error recovery and decision-making processes. Our findings will help formulate methods not only for evaluation of surgical performance, but also for targeting specific skill deficits in surgical trainees.

Methods to train a Kalman filter to decode body motions into the control of a 2D cursor

Despite progress in the field of assistive devices for people who suffered a spinal cord injury (SCI), most of the current devices to control computers and wheelchairs are set in place to require as little physical effort from the user as possible, and little attention has been paid to maintaining and strengthening the neural and muscular resources that survived the injury. We recently developed a body-machine interface (BMI) that allows users to control a 2D cursor by engaging the majority of the motor system that remained intact after paralysis. A calibration paradigm where unimpaired human subjects followed a cursor on a screen as if they were controlling it with their shoulders was used to generate a map between shoulder motions and cursor kinematics. This map was based on Kalman's method for state estimation and we used it to derive the desired cursor coordinates from upper-body motions. Although this study demonstrated promising results on 2D cursor and virtual wheelchair control by a BMI, the design of the experiment instructed the same specific movements in the calibration phase for all subjects in order to reduce variability introduced by different body-to-cursor maps. People with high-level SCI will have individual-specific residual movements, so in the application of this BMI,
they must have the ability to choose their own movements to control the cursor. We expect that
the chosen map will have an effect on task performance. However, we still don’t understand
what characteristics make it a good map vs. a poor map. In this study, we instructed subjects to
follow the cursor with their shoulders using three distinct methods. After familiarizing
themselves with each map, subjects performed one block of a center-out reaching task. We
determined how the different characteristics of each map (orthogonality, reconstruction, mutual
information) correlated with different performance metrics (movement time, variability, path
length, minimum jerk) during the reaching task. Having a method that allows us to predict
whether a subject’s chosen map will result in good performance is advantageous to the subjects
and their therapists. This knowledge allows us to make recommendations to the subject based on
their residual ability that will maximize their performance. Moreover, having a clear
understanding of the relationship between a map and performance can serve as a test bed for the
field of body and brain-machine interfaces to experiment on alternative mapping algorithms and
predict their performance offline.

Disclosures:  I. Seáñez-González: None. C. Pierella: None. E. Thorp: None. A.
Farshchiansadegh: None. F. Abdollahi: None. F. Mussa-Ivaldi: None.

Poster

521. Reaching and Motor Learning

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 521.07/U41

Topic: D.17. Voluntary Movements

Support: NIDRR H133E120010

Title: A predictive model pointing to speed for post-stroke neurorehabilitation

Authors: *Y. ABDEL MAJEED¹, J. L. PATTON²³;
¹Bioengineering, Univ. of Illinois At Chicago, Chicago, IL; ²Bioengineering, Univ. of Illinois at
Chicago, Chicago, IL; ³Sensory Motor Performance Program, Rehabil. Inst. of Chicago,
Chicago, IL.

Abstract: We took up the challenge of using robotic devices to assess and predict clinical
recovery after stroke. Our lab proposed a new method for teleoperation through a bimanual task
that uses the healthy arm to train the affected arm. We extracted features from the point-to-point
reaches and ran a forward model-selection algorithm, which considered these features along with
all demographic information we had on the first day of the study. Our goal was to predict the
change in their clinical performance (as measured by their Fugl-Meyer motor ability score). Our model results show that there is an affinity to maximum speed as the feature of choice for predicting changes in clinical performance. Further analysis revealed that maximum speed was negatively correlated, so that patients moving at lower speeds on the first day tended to have higher Fugl-Meyer change. Such multifeatured models not only provide prognostic tools, but also may also guide therapy by shedding light on the areas that may improve the most.

Disclosures: Y. Abdel Majeed: None. J.L. Patton: None.

Poster
521. Reaching and Motor Learning

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 521.08/U42

Topic: D.17. Voluntary Movements

Support: NIH Grant 5R01EB019834

Title: Effect of intermittent isometric contractions on transcranial direct current stimulation induced alterations in force generating capacity

Authors: *E. P. WASHABAUGH, IV1,2, L. SANTOS1, E. CLAFLIN1, C. KRISHNAN1,2,3; 1Physical Med. and Rehabil., 2Col. of Engin., 3Col. of Kinesiology, Univ. of Michigan, Ann Arbor, MI

Abstract: Transcranial direct current stimulation (tDCS) is a noninvasive brain stimulation technique that is capable of altering motor cortical excitability in a polarity-dependent fashion. Our previous work has shown that a single bout of anodal tDCS significantly increases the force generating capacity of the upper-extremity muscles. Similar findings have been reported in the lower extremity, showing enhanced force production of knee extensor muscles following anodal tDCS. However, when tDCS is concurrently combined with another excitability-increasing event, such as motor practice, interference (aka metaplasticity) may occur that hinders tDCS dependent neuroplastic effects. In this study, we explored the interaction and longitudinal effects of anodal tDCS on force production when paired with a motor learning task. We also tested the time-course of interference following tDCS intervention. For the experiment, nineteen healthy subjects participated in two experimental sessions that were separated by a week. On one session subjects received conventional anodal tDCS over the quadriceps hotspot (determined using transcranial magnetic stimulation) while they were at rest, while during the other session they received tDCS paired with a motor learning task that required them to intermittently perform a
low-level (5% of maximum) force matching task using their knee extensors. The order of the testing conditions was randomized a priori. In both sessions, the subject’s maximal voluntary isometric torque of the knee extensor muscles was measured both before and for up to a half hour following the stimulation using a biodex isokinetic dynamometer. Our preliminary results indicate that, when tested immediately following tDCS, a low-level force matching task interferes with the normal force enhancing effects of anodal tDCS, resulting in a 9.3 N-m decrease in torque output when compared with conventional tDCS. However, this interference effect was short-lived and subjects started to show a trend towards greater torque production within 10 minutes after stimulation. At around 25 minutes, there was virtually no difference in the tDCS mediated changes in maximum voluntary torque values between the two conditions (2.0 N-m). These results suggest that a low-level motor training task during tDCS can interfere with the force enhancing capability of anodal tDCS; however, only for a short-period of time.

Grant: NIH# 5R01EB019834

Disclosures: E.P. Washabaugh: None. L. Santos: None. E. Claflin: None. C. Krishnan: None.

Poster

521. Reaching and Motor Learning

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 521.09/V1

Topic: D.17. Voluntary Movements

Title: The "queen" computational model: learning new distributions through costly encounters

Authors: *A. K. SHAH¹, J. L. PATTON²;
¹Univ. of Illinois @ Chicago, Chicago, IL; ²Sensory Motor Performance Program, Rehabil. Inst. of Chicago, Chicago, IL

Abstract: Movement variability can be a problem in human performance, especially in the recovery following stroke or other neural trauma. Excessive variability can cause people to exceed task limits and cause danger or discomfort to the individual, such as falls (Hausdorff et al., 2001), accidents in the workplace (Branton, 1970) or other costly events. As a result of neural trauma, people need to learn and shift their distributions of movement patterns to avoid costly events because their limits have been altered. Few models predict the learning process and how humans reshape their distributions from costly encounters. Here we present a novel motor controller inspired by the Multi-Armed Bandit (Koulouriotis and Xanthopoulos, 2008) paradigm of explore and exploit. In this case, we use the principle of explore and avoid. This motor
controller develops a motor plan to probabilistically avoid prior experienced regions of high cost that are represented using radial basis functions (Atkeson et al., 1997). We compare the controller’s performance in an environment similar to that of healthy subjects who performed a complex interception task in a recent experiment. During the middle portion of the experiment, we created a high-cost and zero cost region of space to move. Subjects reacted by constraining their distribution of movements within the zero cost region. Our motor controller produces behavior similar to that of subjects who perform this task and other tasks when exposed to the invisible boundary constraint. The model predicts distributions and learning. With further development, this controller can form a new class of motor controllers for robotic control and predictions of human control.


Poster

521. Reaching and Motor Learning

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 521.10/V2

Topic: D.17. Voluntary Movements

Support: University of Michigan Fostering Innovation Grants

University of Michigan Office of Vice President Seed Grant

NIH Grant 5R01EB019834

Title: Optimal design of a passive planar multi-directional resistive robot for upper-extremity rehabilitation

Authors: A. GWOZDZIOWSKI\textsuperscript{1,2}, E. P. WASHABAUGH\textsuperscript{1,2}, C. D. REMY\textsuperscript{2}, *C. KRISHNAN\textsuperscript{1,2,3};
Abstract: Many patients with brain injury (e.g., stroke) experience debilitating loss of upper-limb function. Several lines of research indicate that a large amount of goal-directed reaching movements are critical to promote brain plasticity and recovery. Conventional therapy, however, is often limited due to decreasing third-party coverage. Rehabilitation robots can assist in meeting the increasing demand for therapy; however, existing robots are often large and expensive. Further, because of the importance of muscle strength in recovery of motor function, portable robots that are capable of providing functional strength training could greatly amplify therapy. The portability and safety of a resistive robot can be enhanced if a dissipative passive damping system could be used instead of active actuators. However, there are many possible options for planar manipulators - though, there is an inherent trade-off between design complexity (i.e., number of links) and steerability. Hence, the purpose of this study was to perform a theoretical analysis verified with simulations to obtain an optimal solution for a passive 2D planar resistive robot. We first examined a 2-link 2R planar manipulator with magnetic braking using theoretical analysis derived from the principle of virtual work. Our analysis indicated that an exact solution was impossible due to the inability to achieve independent control of the x and y components of the resistive force. We then performed a simulation analysis to simulate the forces experienced during a straight-line trajectory reaching in order to determine the least-error solution. We found that the angle between least-error and desired forces was substantially large (frequently ≥ 45°), thus ruling out the possibility of a single 2R manipulator. We then examined a 4-link 4R planar manipulator consisting of two 2-link 2R manipulators connected at the end effector. This model was chosen due to its improved steerability, as demonstrated by Gao et al. We determined a system of equations relating the joint braking to the workspace force field. The braking coefficients were then found using the Moore-Penrose pseudo-inverse of the system. Solutions in the nullspace of the system were then added to the resulting solution until all braking coefficients were positive. Our simulations indicated that the computed braking coefficients provided the desired resistance force across the intended workspace. Our preliminary analysis also indicates that control effort could be improved using additional parallel 2R manipulators. Our results have meaningful implications for the design of passive planar robots. Grants: UM FIGs grant, UMOR grant, NIH R01EB019834

Disclosures: A. Gwozdziowski: None. E.P. Washabaugh: None. C.D. Remy: None. C. Krishnan: None.

Poster

521. Reaching and Motor Learning

Location: Hall A
Title: Real-time feedback of estimated intent in the presence of random disturbances increases operator performance and reduces arm stiffness

Authors: *J. R. Horowitz*¹,², T. Madhavan², C. Massie², J. L. Patton²;
¹Rehab Inst. of Chicago, Chicago, IL; ²Bioengineering, Univ. of Illinois at Chicago, Chicago, IL

Abstract: Unstable and uncertain environments are an everyday problem that affects piloting and input devices in human-machine interaction. Here we experimentally evaluate intent feedback (IF), which estimates and displays the human operator’s underlying intent in real-time. IF is a filter that combines a model of the arm with position and force data to determine the intended position. Subjects performed targeted reaching motions while seeing either their hand position or their intent estimate as a cursor while they experienced white noise forces rendered by a robotic handle. We found significantly better reaching performance during force exposure using the estimated intent. Additionally, IF reduced estimated arm stiffness to about half that without IF, indicating a more relaxed state of operation. While visual distortions typically degrade performance and require an adaptation period to overcome, this particular distortion immediately enhances performance. In the future, this method could provide novel insights into the nature of control. IF might also be applied in driving and piloting applications to best follow a person’s desire in unpredictable or turbulent conditions.

Support: NIH R01NS053606-05A1

Title: Customized force field training based on stroke survivors’ individual movement distributions

Authors: *Z. WRIGHT*1,2, J. L. PATTON1,2, F. C. HUANG2, E. LAZZARO2;
1Bioengineering, Univ. of Illinois At Chicago, Chicago, IL; 2Sensory Motor Performance Program, Rehabil. Inst. of Chicago, Chicago, IL

Abstract: Variation in upper extremity motor impairments among stroke survivors creates challenges for the design of robot-assisted therapies. One approach to enhance treatment is to customize based on individual assessments of motor capabilities. However, current strategies are limited by the use of traditional assessments (e.g. Fugl-Meyer, goal-directed performance) for informing customization. Our approach characterizes typical motor behavior through distributions of self-directed motor exploration. We then design unique force fields that push participants towards their neglected movements in the velocity domain. In this study, we investigated how stroke survivors’ (n = 6) movement patterns evolve with customized force field training and compared this to a control group that trained without forces (n = 6). Our results showed that both training groups improved Fugl-Meyer UE scores (2.5 ± 1.0 point and 1.5 ± 0.7 point improvements for the force field group and control group, respectively) and increased their movement capabilities in the velocity domain (104.1 ± 28.1% and 169.8 ± 101.4% increases for the force field group and control group, respectively). These results provide preliminary evidence that patient-specific force fields could be developed into a treatment that expands movement capabilities. To our knowledge, this study is the first to directly link assessments to therapy.


Poster

521. Reaching and Motor Learning

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 521.13/V5

Topic: D.17. Voluntary Movements

Support: NIDRR grant H133E120010

NIH/NICHD grant 1R01HD072080

Italian Ministry of Foreign Affairs, Unit for S/T cooperation
Title: A computational model of learning in a body-machine interface

Authors: *F. A. MUSSA-IVALDI*1,2, C. PIERELLA1,3, M. CASADIO3;
1Rehab Institute of Chicago, Chicago, IL; 2Northwestern Univ., Chicago, IL; 3Univ. of Genoa, Genoa, Italy

Abstract: In the human motor system there is an evident imbalance between the large number of degrees of freedom available to control a particular movement and the smaller number of variables that are needed to specify and plan that movement. Body-machine interfaces (BMI) display the same type of imbalance as they map redundant set of movement related signals into control variables. Redundancy provides the disabled BMI users with the opportunity to identify a comfortable and natural subset of their residual motion abilities that is optimal, or at least adequate, to operate an external device. Understanding the mechanisms that lead to motor function reorganization when subjects learn to use a BMI is crucial to improve its design in terms of usability and adaptability.

We used a state-space model to represent how the interface users through practice learn to form an inverse internal model of the BMI mapping. This internal model is the user’s representation of the cursor-to-body map, that is a right-inverse of the map implemented by the interface from body configurations to cursor positions. The BMI performs a dimensionality reduction by mapping different body configurations into the same cursor position. Hence, users face the task of learning an ill-posed inverse problem. We represented the inverse internal model used by the subject at any time as a state vector that changes under the influence of the current reaching error in the low-dimensional task space. At every reaching block we calculate the gradient of the reaching error and with that we update the state. Preliminary results show that the data simulated with the model and the empirical data have a similar trend. In particular, we focused on the error in the task space, calculated as the distance of the cursor after 1 second from the target to reach, and the similarity of the inverse map with the forward map, calculated as the Euclidean norm of their product. The error in the task space decrease as the subject performs more reaching movements. Concurrently, the internal model of the subjects becomes more stable. Understanding the dynamics of human motor learning in a BMI is of critical importance for co-adapting the interface to the individual evolving abilities of its user.


Poster

521. Reaching and Motor Learning

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 521.14/V6
Title: Evaluation of the efficacy of body machine interface for rehabilitation of spinal cord injury survivors: a pilot study

Authors: *C. PIERELLA*¹², A. DE LUCA², F. CERVETTO², E. TASSO³, S. GAMBA³, L. LOSIO³, A. VENEGONI³, S. MANDRACCIA¹, I. MULLER³, A. MASSONE³, F. A. MUSSA-IVALDI¹, M. CASADIO²; ¹Sensory Motor Performance Program, Rehabil. Inst. of Chicago, Chicago, IL; ²Dibris, Univ. of Genoa, Genoa, Italy; ³Unità Spinale Unipolare, Ospedale Santa Corona, ASL2 Savonese, Pietra Ligure, Italy; ⁴Biomed. Engin., Northwestern Univ., Chicago, IL

Abstract: A body-machine interface (BMI) is a powerful instrument that allows people with severe motor impairments to access assistive devices, perform functionally relevant and/or entertaining activities while concurrently focusing on the achievement of rehabilitative goals. BMIs map the user’s residual mobility onto a control signal for external devices, such as a cursor on a computer screen. The dimensionality of the signals coming from the body is higher than the one of the device control variables. This is a critical feature that allows us to combine assistive and rehabilitative purposes because the map we created between body and device spaces is not unique and it can be modified in order to gradually push the subjects to modify specific characteristics of their movements. Previous studies showed improvements in the clinical tests used to measure strength and mobility at the upper body of spinal cord injured (SCI) survivors. However, the instrumental evaluations to quantify changes occurring pre and post rehabilitative treatment are still limited. Here, we present a method for better characterizing the residual motor abilities following a spinal cord lesion and a possible technique to evaluate motor improvements after a rehabilitation treatment based on the use of the BMI. The evaluation method consists on acquiring kinematic and electromyographic (EMG) data from the upper body during the execution of some functional tasks, like reaching for an object placed at different locations in space, or moving an object between two locations. In this preliminary study we recruited two cervical SCI subjects and 2 control subject of matching age. Only SCI subjects practiced with the BMI 3 times per week, for 5 weeks. From the preliminary data we evaluated task completion time, movement speed and smoothness. We were also able to identify strategies that subjects used to perform different tasks using particular muscle activation patterns, before and after the BMI treatment. After the training SCI subjects improved in the functional tasks in terms of smoothness and completion time. They also exhibited a change in muscle activations used to execute the tasks. While we should emphasize again that these are preliminary observations, the patterns of EMG activations in SCI participants at the end of training had a higher similarity with
those seen in the control subjects. The kinematic and EMG analysis used in this study allowed us to identify and describe some strategies used by SCI subjects during the completion of functional tasks. Moreover it offered a tool for a deeper evaluation of possible changes and improvements that can occur during a rehabilitative treatment through the use of the BMI.


Poster

521. Reaching and Motor Learning

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 521.15/V7

Topic: D.17. Voluntary Movements

Support: 1R01HD072080

Title: Kinetic energy optimization in object manipulation

Authors: *A. FARSCHIANSADEGH1, R. RANGANATHAN2, F. MUSSA-IVALDI3; 1Rehabil. Inst. of Chicago, Chicago, IL; 2Michigan state university, East Lansing, MI; 3Northwestern Univ., Chicago, IL

Abstract: Studies of reaching movements in the horizontal plane have found that straight line trajectories are persistent motor behaviors that are robust to different kinematic and dynamic perturbations. The results of these studies suggest that there are kinematic costs associated with the trajectory formation. However, in the majority of these studies a circular cursor was employed to represent the system under control and the dynamic perturbations were typically in the form of force fields. Here, we suggest that such a simple and symmetric visual representation creates a strong bias towards Euclidean trajectories. We asked human subjects to reach different targets while holding a simulated mechanical linkage. The mechanical properties of this system were set so that the paths of least action were substantially different from straight line paths. Our results demonstrate that subjects exploited the passive dynamics of the manipulated object and moved along curvilinear trajectories that matched the path of least action, which was also the path of minimum kinetic energy of the manipulated object. However, this behavior was contingent upon receiving concurrent and consistent visual and haptic information of the system under control. When either form of feedback was absent or when visual and haptic information
were not mutually consistent, subjects reverted to generating the default pattern of smooth and rectilinear movements.

**Disclosures:** A. Farshchiansadegh: None. R. Ranganathan: None. F. Mussa-Ivaldi: None.

**Poster**

521. Reaching and Motor Learning

**Location:** Hall A

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**Topic:** D.17. Voluntary Movements

**Support:** NIDRR Grant H133E120010

NIH/NICHD Grant 1R01HD072080

**Title:** Spinal cord injury survivors take control with a novel Body-Machine Interface

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**Abstract:** This study tested the use of a customized body-machine interface (BMI) for enhancing functional capabilities in persons with cervical spinal cord injury (cSCI). The interface allows cSCI survivors to operate external devices by reorganizing their residual motion abilities. Eight cSCI participants wore a custom-made garment with motion sensors placed on the shoulders. Signals derived from the sensors controlled a two-dimensional computer cursor. Principal component analysis was used to extract the combinations of sensor signals that best captured each participant's capacity for controlling a computer cursor. Participants practiced with the BMI for 24 sessions over 15 weeks, by practicing three tasks: reaching, typing and game playing. Learning and performance were evaluated by quantifying the movement errors, smoothness and timing, in addition to performance metrics specific to the game and typing tasks. Through practice, participants were able to reduce the movement time (p = 0.002) and the final error (p<0.001) in the reaching task. All participants became more skilled in the typing task and in game playing, as the pong hit rate (p = 0.006) increased significantly with practice. Cursor trajectories became shorter and straighter -i.e. smoother- in most participants. The results provide
proof-of-concept for the customized BMI as a means for people with absent or severely impaired hand movements to control assistive devices that would be normally manually operated.


Poster

521. Reaching and Motor Learning

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 521.17/V9

Topic: D.17. Voluntary Movements

Title: Differences in motor skill learning across lifespan

Authors: *M.-H. Lee¹, A. FARSHCHIANSADEGH²;
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Abstract: When is the best time to learn a new motor skill? Although it is commonly thought that children have superior abilities when it comes to learning skills, this question is difficult to answer using real-world motor tasks because differences in learning abilities are also confounded by physical differences in body size and body composition. Therefore, we sought to minimize these physical confounds by using a novel virtual task and investigate if there were differences in learning a novel motor task across lifespan. We examined differences in learning to operate a novel body-machine interface (BoMI) task across a continuum of the lifespan -- between 9 and 75 years of age. Inertial measurement units were attached to the upper body and these movements were mapped to the position of a computer cursor. The goal of the participants was...
to learn to move the cursor in a center-out reaching task to 8 different targets. Given that the body space is higher dimensional than the task space, we used principal component analysis (PCA) to map the first 2 principal components to the task space. Importantly, this mapping ensured that each participant was capable of doing the task with his or her own movement abilities. There were 160 trials in total toward 4 targets during practice session. Three generalization tests were interleaved during learning (pre, during and post-practice), where participants reached toward all 8 targets. These tests were used to evaluate performance and learning with practice. Overall, the results showed an inverted-U shape with the young adults showing the best performance. Children and elderly tended to have longer movement times (~2x longer) compared to young adults. The analysis of trajectories also showed that children and elderly had less ability to control the cursor, and that children and elderly participant also exhibited greater variability between individuals, which was manifested in the large within group variance. Overall, these differences suggest that young adults are better at learning a new motor task compared to both children and older adults, and this may reflect the time course of development of neural mechanisms for learning.

Disclosures: M. Lee: None. A. Farshchiansadegh: None.
when facing this redundancy is not totally clear. One current hypothesis is that people choose movement patterns that maximize task performance in the face of motor noise. Many studies have demonstrated that motor noise is signal dependent, and thus scales with increased contractions. Theories such as the minimum intervention principle suggest that people select movement patterns that are optimal with respect to minimizing the motor noise that directly impacts task performance, while ignoring motor noise which is irrelevant to the task. Here, we asked the question whether we could use artificial signal dependent noise to shape peoples’ control policies when learning a novel redundant motor task. Subjects learned to control the orientation of their two hands, as measured by roll and pitch of each hand, to control a two dimensional cursor on a screen. They were required to make coordinated hand movements that would move the cursor between specific target locations. During the learning process, we imposed artificial signal dependent noise on each of the input channels. Specifically, the variance of the noise imposed on each input channel was proportional to the magnitude of the signal from that channel. Thus, different movement patterns produced differing levels of cursor noise, which directly affected task performance. This paradigm allowed for the calculation of a single optimal policy, the movement pattern that minimized cursor noise. We found that even in the presence of artificial signal dependent noise, subjects were able to learn to control the interface quickly and effectively, as demonstrated by decreased movement time and increase smoothness through training. We did not, however, find that all subjects converged to the optimal control policy during learning. Overall this work presents a framework for looking at how individuals select an appropriate control policy when learning to resolve redundancy in a novel motor task.

**Disclosures:** E.B. Thorp: None. F.A. Mussa-Ivaldi: None.

**Poster**

**521. Reaching and Motor Learning**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 521.19/V11

**Topic:** D.12. Kinematics and EMG

**Title:** The dynamics of kinematic, kinetic and electromyography parameters of competitive exercise bench press powerlifting in athletes with disabilities

**Authors:** *A. B. TREMBACH, D. LEVCHENKO, I. FEDOROVA, I. PAVELYEV, I. KOMLEV, Y. SHKABARNYA;*  
Univ. of Physical Educ., Krasnodar, Russian Federation
Abstract: Aims: Spatial-temporal parameters of competitive exercise bench press powerlifting is an objective indicator of sport technique and are due to the specifics of the pathology in athletes with disabilities. However, the dynamics of the external and internal structure of this exercise is not sufficiently studied. The aim of the study was to structure sequential time intervals of bench press on the kinematic, dynamic and electromyographic characteristics in athletes with disabilities. Methods: The participants were 20 disabled lifter-athletes of high qualification. Biomechanical parameters and EMG of symmetrical M. Pectoralis major, Latissimus dorsi, Biceps and Triceps brachii during bench press were recorded by complex in powerlifting on the basis of power coordinated platforms making an independent ballistograms (kinetic) and video motion (kinematic). Results: An analysis of the dynamics of speed, acceleration, displacement barbell and ballistogram, found that the significant time intervals of exercise were lowering barbell to the chest, hold it and press. Lowering barbell on the chest included 2 periods and 4 phases. The first period - dispersal barbell had two phases: from the beginning of barbell down to the maximum acceleration, ballistogram (1) and from the development of maximum acceleration, ballistogram to the maximum speed of development (2). Second period - brake barbell had two phases: from the development of the maximum speed to the minimum acceleration, ballistogram (1) and from the minimum speed to the termination of the movement of the barbell (2). Hold the barbell on the chest. Bench press had 2 periods and 4 phases too. First period- acceleration barbell had two phases: from the beginning of movement of the barbell up to the maximum acceleration, ballistogram (1) and the maximum acceleration, ballistogram of the barbell to the maximum speed. In the second period - brake barbell had two phases: from of the maximum speed of the barbell to speed up the development of the minimum (1) and the minimum acceleration to lock the barbell at arm's length (2). The values of the spatial-temporal parameters of the bench press mainly due to the activity of M. Pectoralis major, Latissimus dorsi, and were less M. Biceps and Triceps brachii. Maximum values and expressed synchronization of acceleration, ballistogram and square EMG M. Pectoralis major, Latissimus dorsi detected in first period- acceleration barbell. Duration phase and the values the acceleration and ballistogramm efforts in each of them are individual markers that characterize the level of training of disabled athletes and sports results, respectively.


Poster

522. Neuroprosthetics for Limb Control

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 522.01/V12
**Abstract:** Direct cortical control of hand and arm neuroprostheses has long been posited as a key achievement for intracortical brain-computer-interfaces (iBCIs). As part of the Braingate2 Pilot Clinical Trial, Case Western Reserve University / University Hospitals (Cleveland, OH) is investigating the use of iBCIs to control Functional Electrical Stimulation (FES) neuroprostheses for restoring arm and hand movements to persons with chronic high cervical spinal cord injury (SCI). One individual with C4 level SCI was implanted with two 96-channel microelectrode arrays in the precentral gyrus. The participant has demonstrated robust modulation of single-unit and high frequency spike power (> 300 Hz) sufficient to achieve, using a virtual anthropomorphic limb in stereoscopic space, two and three dimensional control of the wrist position, four dimensional control of the shoulder and elbow joints, and one or two dimensional control of pronation/supination, wrist flexion/extension, and hand aperture. We subsequently implanted 16 percutaneous fine-wire electrodes into the contralateral upper extremity for direct
stimulation of muscles of the arm and hand. To enhance muscle resistance to fatigue, we implemented preprogrammed FES exercises patterns for whole hand grasping (lateral grip), elbow flexion/extension, and shoulder horizontal flexion/extension. During FES exercise, expected channel-specific stimulation artifact was observed on the cortical electrodes. We utilized multiple strategies for reducing and/or eliminating the stimulation artifact, including blanking, signal post-processing, and varying the size and location (including surface vs. percutaneous) of the anode. The participant demonstrated cortical control of the percutaneous FES system in performing a grasp force tracking task, as well as cued single-joint and multi-joint arm reaches. These early indicators of success pave the way for a fully implanted iBCI+FES system to restore complete arm movements to persons with chronic paralysis due to high cervical SCI.


Poster

522. Neuroprosthetics for Limb Control

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 522.02/V13


Support: Office of Research and Development, Rehabilitation R&D Service, Department of Veterans Affairs (B6453R, B6459L, A6779I)

NICHD-NCMRR (N01HD53403)
NICHD-NCMRR (N01HD10018)
NIDCD (R01DC009899)
NICHD (RC1HD063931)
NIBIB (R01EB007401)
NICHD (R01HD077220)

Title: Local field potentials in the motor cortex of people with tetraplegia: comparison using unsupervised methods
Abstract: Dimensionality reduction techniques, applied to neural spike trains, have revealed structure in the motor cortex of non-human primates (NHPs, e.g. Yu et al, 2008, Sadtler et al, 2014, Vargas-Irwin et al, 2015). Local field potential oscillations have been shown to contain information about attempted or imagined movements in NHPs (e.g. Murthy and Fetz, 1992, Sanes and Donoghue, 1993) and people with tetraplegia (e.g. Perge et al, 2014). We propose a method to identify frequency bands of interest in local field potential activity recorded intracortically with a multielectrode array placed in motor cortex. First, we compute LFP power across time using multitap spectral analysis. We then apply factor analysis and k-means clustering to these power spectra and compute the distance between clusters in the frequency domain. We apply this method to data collected from nine people with tetraplegia during a 2D point-and-click cursor control task. These include two people with tetraplegia resulting from spinal cord injury (S1 and T8), two people with tetraplegia and anarthria resulting from brainstem stroke (S3 and T2), and five people diagnosed with amyotrophic lateral sclerosis (T1, A1, T6, T7, and T9). The analysis recovers the prominence of the beta band (10-40 Hz) and suggests differences in beta activity between participants. The results point towards new ways to interpret local field potentials in motor cortex, including in comparisons across task contexts and between etiologies of paralysis.

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Poster

522. Neuroprosthetics for Limb Control

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

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Support: Office of Research and Development, Rehabilitation R&D Service, Department of Veterans Affairs (B6453R, A6779I, P1155R)

- NICHD (R01HD077220)
- NIDCD (R01DC009899)
- NINDS R01NS066311-S1
- MGH-Deane Institute
- Stanford Institute for Neuro-Innovation and Translational Neuroscience
- Stanford BioX-NeuroVentures

Title: Multi-day self-calibration of a point-and-click communication BCI for people with tetraplegia

Authors: *B. JAROSIEWICZ* 1,4,2, A. A. SARMA 3,4, J. D. SIMERAL 4,5,2, D. BACHER 3,2, J. SAAB 3,2, B. SORICE 3, C. H. BLABE 7, S. S. CASH 5,12, E. N. ESKANDAR 6, K. V. SHENOY 8,9,10,11, J. M. HENDERSON 7, L. R. HOCHBERG 4,5,12,2;

Abstract: Brain-computer interfaces (BCIs) aim to restore communication and independence to people with severe motor disabilities by translating decoded neural activity directly into control of a computer cursor. However, nonstationarities in recorded brain activity can degrade the quality of neural decoding over time. Periodically interrupting ongoing use of the BCI to perform
decoder recalibration tasks would be time-consuming and impractical. Previously, we showed that typing performance in a self-paced, neurally controlled point-and-click communication interface can be maintained for hours, despite underlying signal nonstationarities, without requiring the user to pause to perform disruptive calibration tasks. This was accomplished by incorporating 3 decoding software innovations that address different aspects of neural signal nonstationarities: feature mean and variance tracking, decoder output bias correction, and retrospective target inference-based (RTI) decoder calibration, which uses data acquired during practical, ongoing BCI use to recalibrate the decoder. The current study extends self-calibration of the BCI to multiple days. On day 1, a participant diagnosed with amyotrophic lateral sclerosis (ALS) in the BrainGate2 clinical trial (participant T6) performed the usual open-loop and closed-loop decoder calibration center-out tasks with presented targets, and then proceeded to self-paced typing. Then, on days 3, 5, 14, 35, and 42, with the aid of feature tracking and bias correction, the participant was able to proceed directly into self-paced typing using the previous session’s last directional and click decoders. The decoders were updated periodically over the course of the day using data acquired during typing, without ever requiring the participant to perform explicit calibration tasks again after day 1. Multi-day self-calibration was also replicated in a 2nd participant with ALS (T9). By eliminating the need for the user to perform daily calibration tasks with prescribed targets, despite nonstationarities in the underlying neural signals, this approach advances the potential clinical utility of intracortical BCIs for individuals with severe motor disability.


**Poster 522. Neuroprosthetics for Limb Control**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program/#Poster#: 522.04/V15

**Topic:** D.18. Brain-Machine Interface

**Support:** Office of Research and Development, Rehabilitation R&D Service, Department of Veterans Affairs (B6453R, A6797I, P1155R)

- NICHD-NCMRR (N01HD10018)
- NICHD (R01HD077220)
Abstract: Background: Brain-computer interfaces based on intracortical recordings (iBCI) have allowed people with tetraplegia to reliably control a computer cursor on a screen and perform actions with a robotic limb (Hochberg et al., 2006, 2012; Simeral et al., 2011; Kim et al., 2011). Long-term goals of this technology include the ability to switch automatically between volitional neural control of assistive technologies and idle time (“asynchronous” BCI). Idle state detection has been examined in EEG-based BCI (Zhang et al., 2007; Hasan et al., 2011; Lee et al., 2015). Here, we report the ability to distinguish motor cortical activity in task-related blocks from idle inter-task periods in an individual with tetraplegia using an iBCI. Methods: The participant in this study (T9) was a 52-year-old man in the BrainGate2 trial with amyotrophic lateral sclerosis. During research sessions, neural signals were recorded from two 96-channel microelectrode arrays (Blackrock Microsystems, Salt Lake City, UT) implanted into his motor cortex. Average multi-unit spike rates were extracted for each channel during centered-out-and-back radial-8 task blocks and during inter-task periods from two sessions (session 1: 2015.04.22; session 2: 2015.04.23). Channels with statistically significant changes in spike rates between task- and inter-task periods were identified (Wilcoxon signed rank test, p<0.01) and further quantified (e.g., spike rate modulation in %). Results: Spike rate modulation between a block and consecutive inter-block period could be visually observed on many channels. Of the 192 channels, 29 (session 1: 17 on lateral array, 12 on medial array) and 56 (session 2: 48 on lateral array, 8 on medial array) showed a spike rate decrease from task block to consecutive inter-block period (mean±std; session 1: lateral array, -15±4%; medial array, -14±3%; session 2: lateral array, -18±5%; medial array, -15±6%). Only 1 (session 1; lateral array) and 4 (session 2; 2 on each array) channels illustrated a spike rate increase (mean ± std; session 1: lateral array, 18±0%; session 2: lateral array, 33±38%; medial array, 10±1%). Conclusion: In an individual with tetraplegia, intracortical multi-unit activity exhibited statistically significant spike rate changes
between iBCI task periods and intervening idle periods. This suggests that the user’s active engagement with the iBCI system could be distinguished from idle periods. Detecting this engagement could provide greater independence and utility using iBCI communication and permit people with severe motor disabilities to asynchronously control assistive devices.

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**Poster**

**522. Neuroprosthesics for Limb Control**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 522.05/V16

**Topic:** D.18. Brain-Machine Interface

**Support:** NIH Grant 1R01HD077220-01A1

- NIH Grant N01-HD-1-0018
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- NICHD (R01HD077220)
- NIDCD (R01DC009899)
- MGH-Deane Institute

**Title:** Comparing coordinate frame representations in human primary motor cortex for control of reaching

**Authors:** *D. YOUNG*\(^{1,2}\), R. F. KIRSCH\(^{1,2}\), L. R. HOCHBERG\(^{3,4,7,5}\), A. AJIBOYE\(^{1,2}\),'\(^{1}\)Case Western Reserve Univ., Cleveland, OH; \(^{2}\)Louis Stokes Cleveland Dept. of Veterans Affairs Med. Center, FES Ctr. of Excellence, Rehab. R&D Service, Cleveland, OH; \(^{3}\)Dept. of Veterans Affairs Med. Center, Ctr. for Neurorestoration and Neurotechnology, Rehab. R&D Service, Providence, RI; \(^{4}\)Sch. of Engin., \(^{7}\)Inst. for Brain Sci., Brown Univ., Providence, RI; \(^{5}\)Dept. of Neurol., Massachusetts Gen. Hosp., Providence, RI; \(^{6}\)Neurol., Harvard Med. Sch., Boston, MA
Abstract: Single neuron activity from motor cortices has been shown to encode various parameters of natural arm movements, including global arm endpoint position, movement direction, movement speed, and joint velocities. Studies of human intracortical closed-loop control have decoded extrinsic Cartesian endpoint velocities for cursor and robotic control (Hochberg, et al, 2012, Collinger, et al, 2013). However, human arm movements may be better represented and/or controlled in a body-centric joint velocity coordinate frame. Additionally, a decoding scheme in joint velocity space is similar to control schemes implemented in current functional electric stimulation (FES) devices, and hence may be more easily implemented into existing upper extremity prosthetics. In this work, we compared cortical control of a multi-joint anthropomorphic virtual arm using a Cartesian endpoint velocity decoder to a joint velocity decoder. We hypothesized that because evidence of both coordinate frames exist in M1 neural activities, the participant would be able to perform closed-loop control of the virtual arm, though with differing rates of learning of the two decoders. One participant with C4 level spinal cord injury was implanted with two 96-channel intracortical electrode arrays in the dominant precentral gyrus. Using 3D visualizations, the participant watched and controlled coordinated virtual arm movements in a standard center-out reaching task. The user practiced controlling the arm for multiple weeks using two separate control schemes, a 3D endpoint velocity and a 4D proximal joint velocity decoding paradigm. Endpoint and joint velocity decoders were each built in open-loop, followed by standard closed-loop recalibration. The participant trained in each decoding scheme separately using multiple workspaces to learn and improve performance. We then tested the user’s ability to control the virtual arm in Cartesian and joint velocity space on the same day and in the same 3D reaching task. Our participant achieved similar performance using both decoders, hitting above 80% of the targets in both decoder interfaces, but had lower path efficiencies when controlling in joint velocity space. Our work suggests that a paralyzed user can control coordinated reaching in closed loop using both 3D endpoint and 4D joint velocity decoding strategies.

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Poster

522. Neuroprosthetics for Limb Control

Location: Hall A

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Program#/Poster#: 522.06/V17


Support: Office of Research and Development, Rehabilitation R&D Service, Department of Veterans Affairs (B6453R, A6779I)
**Title:** Multiple grasp types can be reliably decoded from the precentral gyrus in people with ALS using implanted intracortical electrodes

**Authors:** *D. BRANDMAN*1,2,3, J. SAAB4,3, C. E. VARGAS-IRWIN2,3, S. E. FASOLI4,5, C. H. BLABE5, B. SORICE10, S. S. CASH10,12, E. N. ESKANDAR11, J. M. HENDERS7, K. V. SHENOY8,9, B. JAROSIEWICZ2,6,3, L. R. HOCHBERG6,4,10,12,3;


**Abstract:** The BrainGate2 clinical trial has demonstrated that individuals with tetraplegia can control prosthetic limbs using neural signals processed from implanted electrodes in the precentral gyrus (Hochberg et. al. 2012). This work was recently expanded to high-dimensional prosthetic limb control (Collinger et. al. 2013, Wodlinger et. al. 2015). These studies used continuous decoding strategies for grasp control; however, an alternate approach for grasp decoding would be to discretely decode only the most common grasps used during everyday activities: the pinch, key and power grips. A discrete decoding strategy focused on these relevant grasps would have several benefits. First, such decoding strategies would not only provide a decoded command (i.e. the maximum-likelihood estimate) but also a level of confidence of the command; if a particular grasping action is detected with a high level of confidence, it can be enacted by an external effector without having to continuously control kinematic parameters online. Second, using a discrete decoding strategy could potentially allow releasing a grasp based on a generic hand-opening command vs. a grasp-specific command. The purpose of this experiment was to explore the feasibility of discrete grasp decoding and the potential advantages outlined above.

**Methods:** Two research participants (T6 and T7) with amyotrophic lateral sclerosis (ALS) were implanted with Blackrock microarrays in the dominant precentral gyrus as part of the BrainGate2 clinical trial. In an open-loop block-randomized instructed-delay session paradigm, they were asked to “perform, or attempt to perform” one of four actions: power, pinch, or key grasp, or supination. Results: A total of 737 actions were analyzed for the two participants.
over five sessions. Raw spike rates were analyzed using a Gaussian Naïve Bayes classifier with 5-fold cross-validation. The action performed could be decoded with greater than 95% confidence during the action epoch. It was possible to decode the grasp-release state equally well using either a generic or grasp-specific class. Conclusion: Wrist supination and the commonly used power, key and precision grasps (the most common grasps used for daily activities) can be decoded with high accuracy from the precentral gyrus in humans. Triggering grasping movements based on high-confidence discrete classification of neural signals offers a robust and functional alternative to continuous control of hand kinematics for neuroprosthetic application, which could bring neural control of prosthetic devices closer to clinical utility.


Poster

522. Neuroprosthetics for Limb Control

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

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Title: Low dimensional dynamics of the primary motor cortex during natural locomotion captures kinematic information and improves decoding performance for brain machine interfaces

Authors: *D. Y. XING*, M. AGHAGOLZADEH, D. BRANDMAN, C. VARGAS-IWIRN, W. TRUCCOLO, D. BORTON;
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Abstract: Many brain machine interfaces (BMIs) decode motor action from cortical ensemble recordings and thus may suffer from issues such as over-fitting. Traditionally, the full neuronal population is used as input into the decoder, which may lead to decreased performance when the ensemble includes neurons that contain little information about the decoded variables. Dimensionality reduction can be a useful preprocessing step for preferentially extracting relevant information from large (>50) populations of neurons. Here, we examined how low dimensional dynamics extracted from different statistical procedures such as Poisson linear dynamic system (PLDS) state-space models, principal component analysis (PCA), and spike train similarity space (SSIMS) methods (Vargas-Irwin et. al., 2015) affected neural decoding performance. Specifically, we compared the performance of gait decoding of Rhesus Macaques during a
variety of locomotion tasks. Neuronal activity from the hind limb area of primary motor cortex was recorded via a 96-microelectrode array simultaneously with recordings of hind limb joint positions and muscle EMGs while monkeys walked across a corridor, a ladder, or on a treadmill at varying speeds. Low dimensional dynamics of the neural ensemble extracted by PLDS showed robust cyclical trajectories which were phased locked to the locomotion gait cycles. These state-space trajectories reflected the kinematic trajectories despite variability in task type and speed. In addition, SSIMS differentially clustered specific tasks and different gait phases in a low dimensional space. A linear decoder with history was used to compute expected joint positions, muscle activations, and phase of the gait cycle (stance vs swing) from both full population neural spike trains and reduced dimensionality inputs. Compared to using the full population, low dimensional inputs improved overall decoding performance with increases in R2 of 0.71 in some tasks. This improvement was observed especially during trials where decoding performance based on the full population was particularly poor. In our data, improvement in decoding performance based on low dimensional neural state trajectories reached a plateau at about 8 dimensions. These results suggest that future BMI design may benefit from incorporating dimensionality reduction approaches for neural decoding.


Poster

522. Neuroprosthetics for Limb Control

Location: Hall A

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- NIDCD (R01DC009899)
- NINDS-Javits (R01NS25074)
- MGH-Deane Institute
Title: Decoding grip type from cortical ensemble activity in humans and non-human primates: Improving classification using training data bootstrapping


Abstract: Although human hands are capable of performing a very large variety of dexterous movements, most activities of daily living can be accomplished using a small set of basic grips including power, precision, and key. Formulating high-accuracy neural decoders for these basic grips is therefore an important goal for the development of assistive brain computer interface devices. Performing this type of decoding in a daily-life setting poses a set of unique challenges. Ideally, it should be possible to generate a decoder using a minimum of training data, so the user does not have to go through a lengthy data collection period in order to train or recalibrate the device. We evaluated the performance of linear (linear discriminant analysis) and non-linear (nearest-neighbor) decoding methods applied to spike counts and spike train similarity metrics. For each method we also examined the effect of augmenting the training data using synthetic spike trains derived as follows for each grip category: First, the distribution of spike counts per trial for each unit is fit using a gamma distribution. The number of spikes fired for each synthetic spike train is randomly chosen from this distribution. Once the desired number of spikes is chosen, n times as many spikes are selected from the full set available in all trials with the same grip (following temporal alignment). The spikes are then jittered using normally distributed temporal shifts. Finally, every nth spike is selected (in order to approximate a refractory period). Our test data included cortical recordings from non-human primates performing the three canonical grips as well as human participants in the BrainGate clinical trial attempting to perform the same grips. Our results show that it is possible to achieve high accuracy grip classification using limited training data (with only about 5 exemplars per category) and a small number of single units (around 15). We also demonstrate that it is possible to significantly increase decoder accuracy by using bootstrapping to augment the training data sets when few training exemplars are available. These results are consistent in all datasets examined, demonstrating similar features of grasp-related neural activity in able-bodied NHPs and humans with longstanding paralysis.

Poster

522. Neuroprosthetics for Limb Control

Location: Hall A

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Program#/Poster#: 522.09/V20


Support: NIH-R01-EB008578

Title: Mechanical fatigue testing of an implantable intrafascicular electrode system


Abstract: We have developed an implantable device that uses longitudinal intrafascicular electrodes (LIFEs) to stimulate/record from small groups of fibers in peripheral nerve fascicles in upper-limb amputees. Since the electrodes and leads must maintain functionality when exposed to stresses during routine activities like walking or lifting, mechanical fatigue testing is necessary to assess the long-term reliability before clinical deployment. Our device consists of a stimulator/recorder unit with a 15-wire lead assembly. The assembly consists of a primary sheathed bundle (15 coiled wires) that leads to a trifurcation to form 3 sheathed bundles (5 coiled wires/bundle), each of which further separates into individual wires (LIFEs). Each LIFE is a 23µm insulated Pt/Ir wire with a 1mm long active zone. Using a needle, each LIFE is sewn longitudinally into the fascicle and sutured to the nerve at the entry and exit points. When implanted, high stresses may occur on the primary bundle near the trifurcation, at the point where the individual wires exit the sheath, or at the nerve suture points. Mechanical fatigue at these points could trigger device failure such as breakage of electrode wires or cracks in the sheath or insulation. We have developed equipment and procedures to expose the device to stress conditions that mimic the anticipated stress profiles in the upper arm. One setup imposes bending stress on the lead bundle near the trifurcation while it is under tension. Another setup imposes longitudinal strain on a compliant structure that models the nerve; LIFEs were sewn into the model nerve and anchored using sutures to mimic surgical installation. Electrode continuity was measured periodically and the status of the lead bundles and wires was assessed using a
microscope. Two mechanical fatigue test paradigms were used: a high cycle/low amplitude paradigm to mimic activities such as walking (7.3 million cycles based on a 2-year design life at 10,000 steps/day) and a low cycle/high amplitude paradigm to mimic strenuous activities such as lifting (1.2 million cycles; based on OSHA guidelines). Bending amplitudes of ±15° (low) and ±45° (high) were chosen based on ISO standards for a similar device. Strain amplitudes of 5% (low) and 15% (high) were chosen based on nerve strain studies. To-date, the high cycle individual wire test has reached 5 million cycles and all other tests have been completed. All wires in all samples (n≥3 samples per test condition) have retained electrical continuity and passed visual inspection. These results suggest that this set of leads and fine wires can maintain functionality in the stressful environment of the upper arm. Supported by NIH-R01-EB008578


Poster

522. Neuroprosthetics for Limb Control

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 522.10/V21


Support: NIH-R01-EB008578

Title: Experimental assessment of fitting procedures for a neural enabled prosthetic hand system

Authors: *L. RINCON GONZALEZ*¹, S. S. KUNTAEGOWDANAHALLI¹, J. J. ABBAS², K. W. HORCH¹, R. JUNG¹; ¹Florida Intl. Univ., Miami, FL; ²Arizona State Univ., Tempe, AZ

Abstract: Stimulation of intact peripheral afferents in upper limb amputees can elicit somatosensory percepts, but prosthetic systems that use peripheral nerve stimulation are not yet available for chronic use outside the lab by amputees. To address this gap, we have developed a Neural-Enabled Prosthetic Hand (NEPH) system, which is based on a fully implantable stimulator and longitudinal intrafascicular electrodes (LIFEs) that deliver electrical stimulation to small groups of peripheral nerve fibers. It includes external electronics for transcutaneous wireless communication, custom-designed firmware to determine appropriate stimulation commands, an instrumented EMG-controlled prosthetic hand (force, position sensors), and custom-designed PC-based software for system calibration and testing. In addition, we have designed experimental paradigms for system calibration and testing. One paradigm has the
subject press a pinch grip force sensor (or a position sensor) and use visual feedback to match a
target level that is presented on a screen; the other has the subject use visual feedback to match
the target (as described above) and then reproduce the action (force or position) using the other
hand, but with no visual feedback. In initial experiments, 7 able-bodied subjects performed the
unilateral force task to validate the experimental procedures. The display had a white
thermometer bar (scaled to the subject’s calibrated force range), a moving red bar to indicate the
applied force, and a green horizontal bar (width = 6% range) to show a target value of 0, 25, 50,
75, or 100%. Subjects were instructed to apply force to match the target level and maintain it for
1 second. Across 72 trials, the target alternated between one of the outer targets (0 or 100%,
presented randomly) and one inner target (25, 50, or 75%, presented randomly). Subjects
performed the experiment with their right and left hand separately. Performance was assessed
using success rate, accuracy, and time to reach the zone. On average, subjects had a success rate
of 97% and 95% with their dominant and non-dominant hands, respectively. In addition, it took
subjects 1.4±0.2 sec to reach the target zone. A one-way ANOVA indicated an effect of target
level on acquisition time; no other significant effects were noted. These results demonstrate that
subjects were able to complete the task and the results provide baseline performance data for
comparison with data from amputees fitted with our NEPH system. We plan to fine-tune
elements of the protocol (e.g. trial time, target zone width) and validate the position task and
bilateral tasks on able-bodied participants prior to testing with amputees using the NEPH system.

Disclosures: L. Rincon Gonzalez: None. S.S. Kuntaegowdanahalli: None. J.J. Abbas:
None. K.W. Horch: None. R. Jung: None.

Poster

522. Neuroprosthetics for Limb Control

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#Poster#: 522.11/V22


Support: NIH-R01-EB008578

Title: Evaluation of an implantable intrafascicular electrode System in rodents

Authors: *A. K. THOTA*, S. KUNTAEGOWDANAHALLI, R. SIU, J. ABBAS, R. JUNG;
Arizona State Univ., Tempe, AZ
Abstract: Electrical stimulation of peripheral nerve afferents can elicit sensations of touch and hand opening in upper limb amputees. To deliver meaningful stimuli that are derived from sensor signals on the prosthetic limb, we have developed a Neural-Enabled Prosthetic Hand (NEPH) system. To the best of our knowledge, this is the first system developed for chronic use by amputees that uses implantable direct nerve interface technology that is controlled via wireless communication from prosthesis-mounted sensors/electronics. The NEPH system consists of an implantable stimulator that directly interfaces with small groups of peripheral nerve fibers via longitudinal intrafascicular electrodes (LIFEs), external electronics that digitize sensor signals from an instrumented prosthetic hand, custom designed firmware to determine appropriate stimulation commands, a link for transcutaneous wireless communication with the implanted stimulator, and PC-based software (CustomTouch) for system calibration and testing. It has been designed, developed, validated and verified via bench testing in accordance with FDA and ISO-10993 standards. In this work, an in-vivo rat model was used to test the system capabilities for impedance measurement, and for stimulation pulse specification, sequencing and delivery. For these tests, an implant emulator was externalized and leads were routed percutaneously to LIFEs (25µm diameter Pt/Ir wire with 4µm thick Teflon coating) implanted in rat sciatic nerve fascicles (2 adult male Sprague-Dawley rats). Wireless protocols were used for bidirectional communication between the PC-based software and the implant emulator. The rat was placed on a pedestal with limbs hanging freely. Stimulation responses were monitored using bipolar fine wire intramuscular electrodes in tibialis anterior (TA) and gastrocnemius (GM) and a video based kinematic tracking system. The strength-duration curve for each electrode was established (threshold amplitude at various pulse widths) and fusion frequency was determined by testing pulse trains at frequencies from 10-100Hz. Two channels were programed to activate TA and GM alternatively (burst duration of 110ms and 310ms, respectively; cycle period of 500ms; 100 cycles; inter-burst intervals at 20ms and 60ms, respectively). Stimulation pulse parameters used for rat 1 were: TA 20 µA, 60 µs; GM 30 µA, 40 µs; for rat 2: TA 105 µA, 150 µs; GM 30 µA, 40 µs. Results demonstrated repeatable reciprocal activation of TA and GM. These studies provide and confirmation of NEPH system capabilities for calibration and for stimulation pulse specification, sequencing and delivery.


Poster
522. Neuroprosthetics for Limb Control

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 522.12/V23
Title: Biocompatibility testing of an implantable intrafascicular electrode system in rabbits

Authors: A. THOTA, S. KUNTAEGOWDANAHALLI, K. HORCH, J. ABBAS, R. JUNG;

Abstract: Longitudinal intrafascicular electrodes (LIFEs) have been used to stimulate or record from small groups of fibers within a peripheral nerve fascicle. These electrodes can be used in sensorimotor prostheses or electronic medicine applications that require a highly specific peripheral nerves interface. We have constructed LIFEs from fine wires (23µm diameter Pt/Ir wire with 4µm thick insulation) with approximately 1 mm long active area and a tungsten needle (75µm diameter) that is used to thread the LIFE into a fascicle and then removed after implantation. Here, we report on biocompatibility studies in rabbits that were performed at a GLP certified facility to evaluate the local neural tissue response to LIFE electrodes. The study used 22 rabbits sacrificed at either 4 weeks (n=11) or 12 weeks (n=11). Using aseptic techniques, LIFEs were implanted in the peroneal and tibial fascicle of the left sciatic nerve. Distal and proximal ends of the individual wires were securely sutured to the epineurium with non-absorbable sutures. For surgical control, tungsten needles were inserted into and removed from right sciatic nerve fascicles. Nerve tissue and electrodes were extracted from all nerves without explanting the electrodes. Three tissue blocks (entry, exit and central parts) of both the left and right sciatic nerves were embedded in plastic resin. Two additional tissue blocks (proximal and distal to entry and exit areas) were embedded in paraffin. Cross and longitudinal sections (thickness = 5µm for plastic; 4-6µm for paraffin) from each block were stained with hematoxylin and cosin, and luxol fast blue. Macroscopic and microscopic evaluations were performed by a board certified pathologist and histological analyses were performed according to part 6 of ISO 10993. Macroscopic evaluation showed no indications of erythema, edema, or other adverse tissue responses. Histological evaluation after 4 weeks showed responses that depended on whether the electrode was within the fascicle or between fascicles at the analysis site. Sites with intrafascicular LIFE showed minimal to mild fibrous tissue and very occasional macrophages and sites with an interfascicular LIFE showed minimal to mild or moderate fibrous tissue, low numbers of macrophages and occasional giant cells. Axonal degeneration, traumatic fibrosis, and seroma formation occurred at comparable incidences and severities at the location of LIFE and surgical control sites. In conclusion, other than slight tissue irritation, there were no adverse clinical or neurological effects or microscopic evidence of local toxicity or adverse reactions following implantation of the LIFEs in the sciatic nerve of rabbits.

Poster

522. Neuroprosthetics for Limb Control

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#/Poster#: 522.13/V24


Title: A brainet for whole-body navigation

Authors: *R. SANKARANARAYANI*, P. TSENG, A. YIN, M. LEBEDEV, M. NICOLELIS;

Abstract: Much neurophysiological research has been done over the years on the properties of cortical neurons to represent both self-initiated actions and the observation of actions performed by others. Yet, it is not well understood whether such neuronal representations could be utilized to enable brain machine interfaces (BMIs) to handle concurrent execution and observation. Even less is known about the possibility of using these representations in Brainets (i.e. BMIs comprised of several brains) first developed by our laboratory. Here we demonstrate a Brainet design where wheelchair navigation is controlled by cortical neuronal ensembles simultaneously recorded in two rhesus macaques. In one version of this design, one monkey (driver) is seated on top of a robotic wheelchair while the second monkey (observer) is stationary and observes the wheelchair movements. Since both the driver's and observer's cortical ensembles are directionally tuned to the wheelchair translational and rotational velocity, the activity of both brains can be used to control the wheelchair navigation. In this Brainet control, the driver is motivated by a food reward at the navigation target location, and the observer is similarly motivated by a food reward which it receives when the driver reaches the target. In the second Brainet design, both monkeys drive wheelchairs, and, again, the activity of both brains is integrated to achieve this control. We suggest that such Brainets in the future will be used for clinical applications where a therapist's brain activity is merged with the brain activity of a patient to rehabilitate function lost to neurological injury or disease.

**Poster**

522. Neuroprosthetics for Limb Control

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 522.14/V25

**Topic:** D.18. Brain-Machine Interface

**Support:** NIH Grant 5R01NS073125-05

**Title:** A brainet for cortico-spinal communication

**Authors:** *A. P. YADAV*¹,², M. A. L. NICOLELIS¹,²,³,⁴,⁵;

¹Dept of Biomed. Engin., ²Ctr. for Neuroengineering, ³Neurobio., ⁴Psychology and Neurosci., Duke Univ., Durham, NC; ⁵Edmond and Lily Safra Intl. Inst. of Neurosci. of Natal, Natal, Brazil

**Abstract:** Dorsal Column Stimulation (DCS) is an effective therapy for a number of neurological conditions, including Parkinson's disease and spinal cord injury. Although DCS has been used in clinic for decades, its full potential has not been realized. In particular, there has been no implementation of a therapist-patient interface, where neural signals derived from a healthy brain would control stimulation applied to the spinal cord of a patient. Here we modeled such an interface using a Brainet that interconnected two rats: an encoder and a receiver. While the encoder rat performed a tactile discrimination task with its whiskers, its cortical signals were recorded, processed by a neural decoder and transmitted to the spinal cord of the receiver rat using DCS. In this Brainet, both rats learned to successfully exchange multiple tactile patterns and improve their behavioral performance. As the rats learned the Brainet task, we observed interdependent adaptations in both the encoder and receiver animals, which suggested that a complex plasticity occurred at the Brainet level. Our study demonstrated for the first time a cortico-spinal communication between different organisms. The obtained results suggest that such a Brainet could be used in the future to enable a healthy brain to directly modulate neural activity in the nervous system of a patient, facilitating plasticity mechanism needed for efficient recovery.

**Disclosures:** A.P. Yadav: None. M.A.L. Nicolelis: None.

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**Poster**

522. Neuroprosthetics for Limb Control

**Location:** Hall A
Title: Wireless brain-machine interface operated by freely behaving monkeys

Authors: *M. J. LEE, S. RAJANGAM, L. OLIVEIRA, M. LEBEDEV, M. NICOLELIS; Ctr. For Neuroengineering, Duke Univ., Durham, NC

Abstract: Neurophysiological experiments in nonhuman primates typically utilize a chair restraint, which limits the range of behaviors and does not allow recordings to last longer than 1-2 hours. This is a significant limitation for brain-machine interface (BMI) research, where long-term performance needs to be achieved under natural conditions. To address this problem, we implemented a wireless BMI in two freely behaving monkeys. Monkeys were housed in a large plexiglass enclosure, where they could continuously control a virtual avatar arm through a BMI that processed neuronal ensemble activity recorded from multiple cortical areas and converted it into the avatar movements. The recordings were conducted using our recently developed wireless multichannel system. Activity of several tens of neurons was decoded using continuous neural decoders. Continuous access to the task was provided for a period of up to eight hours during the day. We observed that the prolonged access to the BMI enhanced the behavioral performance. Both monkeys successfully acquired the ability to operate the BMI as they freely roamed about their cage. This learning was accompanied by adaptive changes in cortical modulations to the avatar arm movements. We suggest that such long-term BMI control facilitates an incorporation of the avatar into the brain representation of the body. As such, our wireless BMI is applicable to neurological conditions that require brain reorganization to occur for the treatment to be effective.

Abstract: Previously we have demonstrated visual responses in monkey somatosensory (S1) and motor (M1) cortical areas during a rubber hand illusion (Shokur et al. 2013). Here we explored whether steady-state visual evoked potentials (SSVEPs) could be recorded in the same areas and whether they would be modulated by visual attention. SSVEPs were incorporated into a two-alternative reaching task. In each trial two circular targets were presented on the computer screen. Each targets flickered at a unique frequency (e.g. 9Hz or 13Hz). Non-flickering targets were displayed in 30% of the trials to establish a baseline. Rhesus monkeys manipulated a hand held joystick to reach targets with a computer cursor. Spikes and local field potentials (LFPs) were recorded simultaneously using chronically implanted multielectrode arrays. The neural data were processed using a non-parametric multitaper spectral estimation method to assess frequency modulations at a single trial resolution. Spectral peaks at the flicker frequency were found in both spike and LFP data. These results for the first time show that intracranial recordings from S1 and M1 can be utilized to continuously record visual responses, which are relevant to motor behavior, incorporation of avatar arm, and visual attention. As such, this approach can be useful to build better neural prostheses.

Title: Extracting high-frequency features of neural activity from μECoG surface electrodes

Authors: *C. BARTON¹, S. KELLIS², P. HOUSE³, B. GREGER¹;¹Sch. of Biol. and Hlth. Systems Engin., Arizona State Univ., Tempe, AZ; ²Biol., Caltech, Pasadena, CA; ³Neurosurg., Univ. of Utah, Salt Lake City, UT

Abstract: While non-penetrating surface electrodes tend to provide recording longevity and minimal tissue response, penetrating electrodes must normally be used to record individual action potentials from cortical neurons. However, general spiking activity has been shown to be associated with increased energy from 300 - 6000 Hz in the frequency spectrum (Pezaris, 2000). More recently, surface electrodes have been developed that were able to record isolated spikes with features of superficial cortical neurons (Buzsaki, 2014). Here, we examine data previously recorded from subdural micro-electrocorticography arrays implanted in human patients over the face-motor cortex and Wernicke’s area. Classification of spoken words had previously been achieved by decoding LFP data recorded from these arrays. Several channels also exhibit a small but consistent increase in frequency spectrum energy extending as high as 1 kHz, which could potentially indicate spiking activity. This increase in high-frequency power could represent an additional feature of neural activity that could be extracted from μECoG surface electrodes. By developing a decoding algorithm incorporating these features in addition to lower-frequency spectra associated with LFP, it may be possible to determine whether useful information encoded in higher frequency bands may be detected from μECoG arrays.

Disclosures: C. Barton: None. S. Kellis: None. P. House: None. B. Greger: None.

Poster

522. Neuroprosthetics for Limb Control

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 522.18/V29

Title: Multiclass support vector machine decoding of spoken words from micro-electrocorticography recordings over Wernicke’s area and face motor cortex

Authors: *D. OSWALT¹, S. KELLIS², P. HOUSE³, B. GREGER¹;
¹Sch. of Biol. and Hlth. Systems Engin., Arizona State Univ., Tempe, AZ; ²Div. of Biol. and Biol. Engin., Caltech, Pasadena, CA; ³Dept. of Neurosurg., Univ. of Utah, Salt Lake City, UT

Abstract: Severe motor disorders or brain stem damage can leave individuals severely paralyzed but fully aware and unable to communicate by most natural means. For these individuals a direct cortical interface may provide more rapid and intuitive control over a communication prosthesis. Brain-computer interfaces (BCI) have potential for functional restoration and improving quality of life. Decoding natural cortical patterns for individual spoken words would allow for a system which requires very minimal patient training to maintain a high degree of accuracy. Previous attempts have been successful in using principal component analysis to decode single words from cortical activity at levels above chance, serving as a proof of concept. This study reanalyzes micro-electrocorticography (µECoG) data recorded during a repetitive speech task by applying support vector machines (SVM) to increase word prediction accuracy from local field potential (LFP) features. Two µECoG grids were placed on the cortical surface over language areas (Wernicke’s area and Face Motor Cortex) of an adult male patient undergoing clinical monitoring for medically refractory epilepsy. LFPs were recorded while the patient was instructed to articulate one of ten words with one second intervals. Trials were aligned to the start of articulation. Snippets from the onset of articulation to 500 ms post articulation were extracted for each trial. Three feature sets were created from the snippets, a time domain series, a frequency domain series and a combined feature set created by concatenating time and frequency domain features. Features from 15 trials for each word were used to train the classifier and 15 subsequent novel trials were used for evaluation. Features were classified using a one-vs-one multiclass SVM classifier with radial basis function kernel. The most successful parameters yielded an accuracy of 83.3% in selecting the correct word from ten possible options and an average of 96.3% accuracy for two word combinations. This is an improvement from the 48% accuracy achieved for ten word classes and average 85% accuracy for two word classes reported with principal components analysis decoding [1]. Decoding spoken words from µECoG recordings using SVM performed well above chance and provide addition support for µECoG as a useful neural interface. [1] Kellis, Spencer et al. “Decoding Spoken Words Using Local Field Potentials Recorded from the Cortical Surface.” Journal of neural engineering 7.5 (2010): 056007. PMC. Web. 5 May 2015.


Poster

522. Neuroprosthetics for Limb Control
Title: Shared control system between a robotic arm and user intent decoded from posterior parietal cortex

Authors: *D. BROWN*¹, T. DOBREVA¹, S. KELLIS¹, K. D. KATYAL², M. S. JOHANNES², C. KLAES¹, R. A. ANDERSEN¹;
¹Div. of Biol. and Biol. Engin., Caltech, Pasadena, CA; ²Applied Physics Lab., The Johns Hopkins Univ., Laurel, MD

Abstract: The posterior parietal cortex (PPC) receives sensory information about the location of the body and external objects and outputs planned movement intent. In particular, the anterior intraparietal area (AIP) and Brodmann area 5 (BA5) are responsible for grasp intention and reach movement, respectively. Encoded intention signals from PPC then travel along the motor pathways where the motor cortex develops a more detailed plan for limb movements. Analogously, autonomous robotic systems can integrate sensory data from the environment to determine the detailed kinematics of joint angles necessary to execute high-level commands. Thus, there is a natural fit to use movement intentions from PPC as the high-level commands for a partially autonomous neuroprosthetic. We developed a shared control system that uses decoded PPC intent to guide the movements of a robotic arm. This allows the user to focus on high-level movement intentions, such as grasping or reaching an object, without thinking about the movements of each individual joint. The system includes a Microsoft Kinect for machine vision, a JACO robotic arm as a manipulator, and signals extracted from a neural decoder containing the user's intent. Microelectrode arrays implanted in AIP and BA5 of tetraplegic human subjects supply neural data to the neural decoder. The goal of the system is to identify objects in the user’s environment and perform activities of daily living, such as drinking a bottle of water, with the assistance of the robotic arm. The system uses the Kinect's image and depth data to identify objects and send their location to the robotic arm. The arm proceeds through the trajectory of motions to pick up the object and performs a context-relevant action. The trajectories composing the task are throttled by the confidence of the neural signal associated with the user's intent to perform that action. The shared control system is currently capable of identifying a bottle of liquid, picking it up, bringing it to the user's mouth, tilting it for drinking, and returning it to its
original position. Progression through the different states of the task is controlled by the subject imagining bringing an object to his mouth. The tetraplegic subject being able to drink from a bottle using intuitive high-level intent shows promise for PPC-based shared control neuroprosthetics as a viable means for individuals with severe motor impairment to regain some independence.


**Poster**

**522. Neuroprosthetics for Limb Control**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 522.20/V31

**Topic:** D.18. Brain-Machine Interface

**Support:** NIH Grant EY015545

The Boswell Foundation

**Title:** Hand specificity in human parietal neurons and local fields


**Abstract:** Neural signals in the posterior parietal cortex have been shown to encode, among other movement variables, information regarding the state of the arm. This functionality is presumably supported by its substantial connectivity to somatosensory and visual areas. In one of the first extracellular recordings in the human parietal cortex in a tetraplegic subject (six years post-injury), we observed single units and LFPs that preferentially responded to hand-related movement execution and hand-related visual feedback in offline tasks. In an online brain control task performed in 3D virtual reality, we observed changes in spike activity that preferentially improved control of an anthropomorphic hand viewed from the 1st person perspective but not an abstract, disembodied, cursor-like effector. Our findings strengthen the notion that some of the neural machinery in the superior parietal lobule is specialized for feedback control of the hand. Furthermore, this specificity is retained in human parietal activity even years after the loss of motor control of the limb due to high spinal cord injury.

Poster

522. Neuroprosthetics for Limb Control

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 522.21/V32


Support: National Institutes of Health

Boswell Foundation

Title: Representation of executed, attempted, and imagined actions in a tetraplegic subject and implications for brain-machine interfaces

Authors: *C. Y. ZHANG1, T. N. S. AFLALO1, B. REVECHKIS1, R. A. ANDERSEN1, E. R. ROSARIO2, D. OUELLETTE2, N. POURATIAN3, K. PEJS2, S. KELLIS1, C. KLAES1; 1Caltech, Pasadena, CA; 2Casa Colina Hosp. and Centers for Healthcare, Pomona, CA; 3Univ. of California Los Angeles, Los Angeles, CA

Abstract: We explored the neural representation of executed, attempted, and imagined actions in the posterior parietal cortex of a tetraplegic subject with a C3-C4 spinal lesion in a BMI clinical trial. We studied imagined and executed shoulder movements, actions she uses commonly to control her wheelchair. We also studied the representation of imagined and attempted hand actions, of a body part she is unable to control. We found some neurons tuned more for execution, some more for imagination, and some with comparable/unbiased tuning for both. The unbiased units showed effector specificity, active only for left or right shoulder actions. This suggests that specificity and cognitive strategy are coded independently. The pattern of results were similar when we compared the representation of imagined and attempted hand movements. Given that different populations were activated by different strategies, we asked which strategy, and therefore which population, was best for online brain control. Using a virtual cursor control task we found both to be viable, with the attempt strategy more successful on days with significant performance differences. Neuron dropping curves showed open loop imagine performance to be better than attempt performance. Interestingly, in closed loop control attempt performance improved while imagine performance did not change significantly, a result that makes physiological sense. To understand these differences we looked at how well each neuron controlled the cursor at each time point in online control. We found that performance differences
were driven by the subpopulation of neurons biased to the attempt strategy. Our results suggest that the imagine strategy may be more open loop and unaffected by the feedback, while the attempt strategy handles feedback better. In able-bodied people the difference between executed and imagined actions is clear, with fMRI scans showing overlapping networks for the two strategies. In paralyzed patients, however, it is unclear if the networks involved are still even intact years after injury. Our results confirm the overlapping networks found in the fMRI results, finding them intact even years after paralysis. The results also suggest that cognitive motor strategy is an important factor in brain control and the choice of strategy can impact performance. In therapies imagined motions are seen as a way to train networks when the subject is unable to move for long periods of time. Our results show that attempted actions are still well represented and different from imagined actions. Understanding the benefits of each strategy may be integral in developing a robust BMI as well as improving rehabilitation techniques.


Poster

522. Neuroprosthetics for Limb Control

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 522.22/V33


Support: NIH EY013337

NIH EY015545

Boswell Foundation

USC Neurorestoration Center

Title: Representation of decision variables in the human posterior parietal cortex

Authors: *C. Klaes*¹, S. Kellis¹, T. Aflalo¹, B. Lee¹,², B. Reveckis¹, C. Zhang¹, K. Pejsa¹, K. Shanfield¹, S. Hayes-Jackson³, M. Aisen³, C. Heck², C. Liu², R. A. Andersen¹;¹Biol., Caltech, Pasadena, CA;²USC, Los Angeles, CA;³Rancho Los Amigos Natl. Rehab. Ctr., Downey, CA
Abstract: From Rhesus monkey studies it is known that the posterior parietal cortex (PPC) is involved in decision making and reward processing. In our current human brain-machine interface study we had the opportunity to investigate the involvement of the human PPC in decision making while playing a two player press-your-luck game. Our study participant was implanted with two Utah electrode arrays (UEA), one in a grasp related area (anterior intraparietal area) and one in a reach related area (Brodmann area 5). The subject played a simplified two player variant of Black Jack while we recorded brain activity. In this game a series of numbers is presented to the first player sequentially and each time a decision has to be made to either proceed ('hit') or stop ('stay'). The first player’s turn ends if the player decides to stop proceeding or the sum of all previous numbers exceeds a certain limit. Afterwards a second player takes his or her turn in the same way. After both players finish their turn the player who was closest to the limit but did not exceed it wins the game round. Our preliminary results indicate that a population of neurons in the PPC tracks the cumulative score during the game. Interestingly we also found neurons which keep track of the opponent's cumulative score. Besides tracking score we could also find neurons that reflect the upcoming decision near the end of a trial. These preliminary findings give a detailed outlook at various variables involved in decision making and how they are represented in the PPC.

Abstract: Tuning curves have been traditionally used to quantify how single units represent limb movement covariates. In doing so, however, they ignore the temporal dynamics of a unit’s firing rate, making it hard to quantify the extent to which changes in these dynamics may reflect representations of other covariates in the task space. In the context of brain-machine interfaces (BMIs) when no overt natural arm movements are produced, neural decoders are an example of such covariates where simultaneously recorded units are individually weighted by the decoder filter coefficients to extract the desired kinematic variable in near real-time. Maximum output of the decoder is expected when the dynamics of unit firing rates match those of the decoder filter. However, since different firing rate patterns can give rise to the same kinematic pattern, it is unclear whether subjects exploit this strategy when volitionally controlling motor intent signals during BMI operation. In this work, we analyze multiple single-unit firing patterns from a chronically amputated adult female rhesus macaque during the control of a robotic arm in a reach-to-grasp task. Hand velocity and aperture velocity decoders were initialized from spontaneously active neurons using a non-biomimetic approach and held fixed throughout all training sessions. We examined those patterns immediately preceding (1 sec) and following (1 sec) moments when the robotic hand velocity command hit its maximum allowable limit. Using this event, we constructed Peri Event Time Histograms (PETHs) for all the single-units (n=9) that drove the hand velocity decoder. These PETHs were constructed from 20 single day sessions of closed-loop BMI spanning two calendar months. We found that the PETHs of all of the units qualitatively exhibited characteristic shapes during these intervals. Moreover the PETHs of 44% of the units were significantly correlated with the corresponding decoder filter dynamics (p<0.05). This evidence suggests that BMI subjects employ a consistent control strategy that matches the characteristic shapes of linear BMI decoders.

Poster
522. Neuroprosthetics for Limb Control

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 522.24/V35


Support: NIH U44 NS067784
DARPA HR0011-15-C-0036

Title: Wireless multichannel implant for neuromuscular interfaces

Authors: *D. MCDONNALL, C. SMITH, D. MERRILL, S. GUILLORY, S. HIATT;
Ripple, Salt Lake City, UT

Abstract: Control of prosthetic arms has been limited by the small number of inputs that are used to control multiple degrees of freedom in the limb. Conventional myoprostheses rely on a pair of signals recorded from surface electrodes on the residual limb. We are developing an implantable multichannel myoelectric device to detect signals from multiple residual muscles that will be sent wirelessly to the prosthetic limb. This approach offers the advantages of recording more channels of isolated muscle signals and providing access to deep muscles that cannot be detected with surface electrodes. We report the results of a proof-of-concept study to verify the in vitro performance of the system and an in vivo trial to validate device function in an animal model. The implant was constructed on a ceramic circuit board with a bioamplifier ASIC and additional discrete components. The implant was inductively powered by an external transceiver, and digitized signal data were sent from the implant by reflected impedance modulation. Each implant included four pairs of electrodes in epimysial disc, intramuscular bands, and fine wire configurations. The electronic components and ASIC die were coated with a conformal electronics sealant, and the entire assembly was coated in silicone. Benchtop implant performance was verified in a dry configuration and while the devices were soaked in saline. The amplifier was shown to have an input-referred noise of 2.2 µVRMS, a common mode rejection ratio greater than 55 dB, and neighboring channel isolation averaging 66 dB. The system uses an infrared telemetry approach to transmit high-bandwidth transmission (10Mbps) of detected signals. Emitters used for these experiments typically operated with a 30% to 40% power to light conversion efficiency, and optical recovery at the receiver was typically on the order of 0.5% to 5%. These prototype implants were validated in a six-dog study at the University of Utah. Two four-channel devices were implanted bilaterally in the front limb by placing the electronics package behind the shoulder blades with electrodes implanted in deltoideus and lateral head of
triceps. One week following implantation, each animal was fitted with a backpack carrying an external transceiver coil and a battery-powered data acquisition system, and the dogs were allowed to freely walk down a hallway. EMG recorded from each animal as it walked down a hallway had very low noise and, in conjunction with recorded video, clearly indicated swing/stance phases of gait.

**Disclosures:**

- **D. McDonnell:** A. Employment/Salary (full or part-time); Ripple LLC.
- **C. Smith:** A. Employment/Salary (full or part-time); Ripple LLC.
- **D. Merrill:** A. Employment/Salary (full or part-time); Ripple LLC.
- **S. Guillory:** A. Employment/Salary (full or part-time); Ripple LLC.
- **S. Hiatt:** A. Employment/Salary (full or part-time); Ripple LLC.

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**Poster**

**523. Stress: Factors Affecting Sensitivity, Protection, and Recovery**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 523.01/V36

**Topic:** E.05. Stress and the Brain

**Support:** CDMRP Grant W81XWH-09-2-0098

Intramural Funds from Centers for Disease Control

**Title:** Corticosterone primes the neuroinflammatory responses to Gulf War Illness associated exposures: Effects of irreversible vs. reversible acetylcholinesterase inhibitors

**Authors:**

- Mol. Neurotoxicology, CDC/NIOSH, Morgantown, WV

**Abstract:** Following the 1991 Persian Gulf War, as many as 250,000 soldiers returned with symptoms of Gulf War Illness (GWI), a complex disorder with characteristics similar to neuroinflammation-driven “sickness behavior.” These troops were exposed to a variety of cholinesterase inhibitors during deployment, including exposures to irreversible organophosphate (OP) acetylcholinesterase inhibitors (AChEI), in the form of the insecticide [chlorpyrifos (CPO)], or to warfare nerve agent, sarin. The reversible AChEI, pyridostigmine bromide (PB), was self-administered by soldiers as prophylactic treatment for potential nerve agent exposures. Alongside exposure to these AChEIs in the Gulf War were physiological stressors (e.g., high temperatures, physical exercise, or physical threat). Here, we examined effects of exposure to 3 OPs: CPO, DFP (as sarin surrogate), and PB in mice. These AChEIs...
were given alone and with pretreatment with the stress hormone corticosterone (CORT) to mimic high levels of physiological stress. We assessed effects of these treatments on brain regional neuroinflammation by qPCR and neuroinflammation-associated microglial/astroglial activation by levels of phosphorylation of STAT3 (tyr705). Adult male C57BL/6J mice were exposed to CORT (400mg/L in 1.2% EtOH), or water for 4 days. On the 5th day, mice were exposed to a single i.p. dose of CPO (8.0mg/kg), DFP (4.0mg/kg), or PB (3.0mg/kg). qPCR at 6 hours post-exposure revealed that acute CPO and DFP treatment alone produced neuroinflammation in the cortex and hippocampus that was enhanced by CORT pretreatment. Furthermore, CORT + CPO or DFP exposure enhanced activation of STAT3 in both brain regions. In contrast, acute exposure to PB alone or with CORT pretreatment did not produce significant increases in neuroinflammation or STAT3 activation. Exposure to the irreversible (CPO, DFP) or reversible (PB) AChEI with or without CORT pretreatment did not result in astrogliosis (increases in GFAP) in either the cortex or hippocampus, findings suggestive of an initial lack of neurodegeneration. Overall, exposure to irreversible AChEIs, CPO and DFP, produce neuroinflammatory effects in the cortex and hippocampus that are augmented by CORT pretreatment, an effect not seen with exposure to the reversible AChEI, PB. These effects may be a result of selective CORT priming of the JAK2/STAT3 pathway for specific GWI-related compounds - an effect that does not induce early signs of neurodegeneration. Thus, this study reveals the potential for selective pathway activation by irreversible AChEIs, a stress-hormone primed neuroinflammation in the absence of neurodegeneration that results in symptoms of GWI.


Poster

523. Stress: Factors Affecting Sensitivity, Protection, and Recovery

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 523.02/V37

Topic: E.05. Stress and the Brain

Support: Center Grant: P60- AA011605

Title: Acute stress activates neurons and microglia across multiple brain regions: impact of adolescent intermittent ethanol treatment

Authors: *T. J. WALTER, R. VETRENO, F. CREWS;
Univ. of North Carolina - Chapel Hill, Carrboro, NC
**Abstract:** Stress affects the brain in many ways, impacting neurons as well as microglia, the resident macrophages of the brain. Furthermore, prior experience can change the way the brain responds to stress. Previous studies suggest that alcohol abuse during adolescence may alter either the neuronal or microglial response to acute stress during adulthood. Therefore, this study examined the response of neurons and microglia to acute stress, as well as the impact of prior adolescent intermittent ethanol (AIE) treatment on this response. This was done by treating Wistar rats with alcohol during adolescence and an acute restraint-water immersion stressor during adulthood. The neuronal response throughout the brain was assessed by immunohistochemical staining for c-Fos and EGR1. This was compared to the microglial response, which was assessed by immunohistochemical staining for CD11b. Acute stress increased c-Fos+ cell counts, EGR1+ cell counts and CD11b+ staining across the brain. While AIE did not impact the c-Fos response to acute stress, it did alter the EGR1 response in the CeA. Prior treatment with AIE enhanced the CD11b response to acute stress in many, but not all, brain regions. Neither acute stress nor AIE altered microglial cell number, and staining for markers of advanced microglial activation, such as CD68, MHCII and iNOS, were negative. The stress-induced increase in CD11b+ staining appeared to be associated with a hyper-ramified microglial morphology. These results suggest that acute stress causes hyper-ramified microglial activation across the brain that is enhanced in select regions by prior binge alcohol treatment.

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

**Poster**

**523. Stress: Factors Affecting Sensitivity, Protection, and Recovery**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 523.03/V38

**Topic:** E.05. Stress and the Brain

**Support:** DMRDP, MOMJPC-5

**Title:** Combined electrophysiological and behavioral approaches in evaluation of neuro-toxicity due to repeated jet fuel exposure in rats

**Authors:** *J. G. ROHAN*1,2, M. K. MIKLASEVICH1,3, S. M. MCINTURF1, C. P. GUT, Jr.1,3, K. L. MUMY1;

Abstract: Jet fuel is a typical occupational exposure hazard among civilian and military personnel. Reports have documented significant impairments in attention-based tasks and memory in humans following jet fuel exposures. On the other hand, neuro-behavioral studies performed in animals have reported few neurological effects of prolonged jet fuel exposures, despite significant changes in neurotransmitter levels such as dopamine and serotonin. To date, there are no published data regarding the effects of in vivo jet fuel exposures on neuronal function in intact brain slices. Here, we combined electrophysiology techniques with behavioral approaches to assess and correlate neurological effects due to jet fuel exposure in a rat model. We exposed male Fischer rats to 0 (control), 200 (low), 1000 (medium) or 2000 (high) mg/m³ concentrations of jet fuel (JP-8 or Jet A) using whole-body inhalation chambers for 28 days (5 d/wk, 6 hrs/day). Behavioral assays (acoustic startle response with pre-pulse inhibition and Morris water maze) were performed to assess cognitive effects. Extracellular recordings were performed within 24 hours of exposures, in which we evaluated effects of jet fuel exposures on evoked responses, synaptic plasticity (LTP, LTD, paired pulse facilitation) and spontaneous activity of acutely dissected hippocampal slices. Preliminary findings indicate no dramatic differences in hippocampal function after 28 days of “occupational” in vivo exposures to either JP-8 or Jet A. However, we detected small but significant differences in evoked inhibitory response amplitudes surrounding the CA1 region between the control and medium groups. There was a trending decrease in spontaneous activity surrounding the CA1 region of the hippocampus in rats exposed to the highest concentration of JP-8 or Jet A. In contrast, there was a trending increase in spontaneous activity surrounding the dentate region of the hippocampus in rats exposed to high concentration of Jet A. No changes in synaptic plasticity were observed, even in rats exposed to the high concentrations. Consistent with this finding, there was no significant deviation in spatial learning/memory or sensorimotor gating, as evaluated by the Morris water maze and acoustic startle reflex with pre-pulse inhibition tests, respectively. Data gathered to date suggest that current occupational exposure limits (200 mg/m³) are protective against the neurotoxicity endpoints evaluated. The effects of exposure to very high concentrations of jet fuel, as well as alternative fuels, still warrant further investigation with regard to subtle differences that may occur in humans but not be entirely reflected in the rat model.

Methamphetamine regulates stress-related neuropeptides via diverse epigenetic mechanisms

Abstract: A single methamphetamine (METH) (10 mg/kg) dose caused time-dependent increases in stress-related neuropeptides, including Crh, Avp and Cartpt in the nucleus accumbens of rats. In general, gene transcription is regulated by complex epigenetic mechanisms that include covalent histone modifications or DNA methylation / hydroxymethylation. Here we show that METH significantly decreased DNA methylation at the CpG-rich sites near the promoter region of Crh and at CpG-rich intragenic sites of the Avp gene. METH caused no changes in DNA methylation but increased enrichment of pCREB on the Cartpt gene promoter. METH-induced hypomethylation is mediated via a cascade that includes DNA hydroxymethylation. METH was shown to increase DNA hydroxymethylation at the CpG-rich sequences located near the Crh TSS and at Avp intragenic sites. Because Ten-eleven translocation (TET) enzymes catalyze DNA hydroxymethylation, we conducted chromatin immunoprecipitation (ChIP) assay using TET1, TET2 and TET3 antibodies and found significant METH-induced increase of TET1 binding on the Crh promoter sequence and of TET3 binding on an Avp intragenic region. There were no changes in TET2 binding on Crh and Avp DNA sequences. Inhibition of TET activity using 1, 5-isoquinolinediol (ISO) blocked the METH-induced Crh and Avp mRNA expression. Together, these results show a potential role of TET in mediating METH-induced up-regulation of Crh and Avp mRNA expression in the nucleus accumbens. These observations hint to the possibility that METH can activate diverse regulatory epigenetic mechanisms to regulate gene expression in the brain.


Poster

523. Stress: Factors Affecting Sensitivity, Protection, and Recovery

Location: Hall A
Title: Choline supplementation to pregnant mice mitigates the neuroinflammatory effects of prenatal diesel exposure to fetal brain

Authors: *S. V. MAURER, J. L. BOLTON, C. E. TYBOUT, S. D. BILBO, C. L. WILLIAMS; Duke Univ., Durham, NC

Abstract: Numerous studies have shown that air pollution causes widespread inflammatory processes in body and brain and is linked to neurocognitive difficulties, increased anxiety and depression, and increased prevalence of neurodegenerative disorders. When exposure to air pollution occurs early in development, children show decreased working memory ability (Sunyer et al., 2015). As well, prenatal exposure to diesel particulate matter increases inflammatory cytokine expression within several brain regions of embryonic day 18 males and leads to long-term negative outcomes for the offspring (Bolton et al., 2012; 2014). In contrast, dietary choline supplementation has been shown to decrease inflammation in adult rats and humans (Rivera et al., 1998; Mehta et al., 2010) and when administered as a supplement to pregnant rats, choline also increases working memory and decreases age-related cognitive decline in the offspring (Meck et al., 2008). The current study sought to determine if dietary choline supplementation protects against the deleterious effects of air pollution on the developing brain. Time-mated C57/Bl6 mice were given a high-choline (SUP – approximately 4.5X choline levels in the CON diet) or control (CON) diet, and a series of diesel particulate (DEP) or vehicle (VEH) exposures throughout pregnancy. Mice were sacrificed and tissues collected on embryonic day 18. The number and activation state of microglia, identified by Iba1+ immunohistochemical staining, in several brain regions were examined to determine the impact choline and/or diesel on microglial development. As expected, we found that the CON/DEP mice had significantly more stout and amoeboid microglia and more total microglia per volume in the dentate gyrus than the CON/VEH and SUP/VEH groups. Remarkably, prenatal choline supplementation to the DEP group completely prevented this increase in microglia number and change in morphology. The effects also appeared to be regionally specific: we found different effects of choline and diesel in fetal hippocampus, fetal hypothalamus, and maternal hippocampus. These findings suggest that prenatal choline supplementation throughout pregnancy may protect the fetal hippocampus against the neuroinflammation associated with air pollution. While we have not yet identified a mechanism of action, choline may be having these effects via an epigenetic mechanism or possibly via direct activation of α7 nicotinic acetylcholine receptors in the developing brain (Wu et al., 2015). Further work is underway to determine how choline supplementation to the pregnant dam leads to alterations in fetal and maternal response to air pollution.
Abstract: We do not know how a hyperthermic exposure, that widely is using in cancer clinics, especially in case of whole body hyperthermia (WBH), can affect the brain functions, and what impact it will have on blood rheology. But we do know that the hyperthermia will cause an oxidative stress, the positive or negative consequences of which will be dependent on what we will get - activation or inhibition of hormetic mechanism Proceeding from the above, we decided that the study the WBH effects on the processes of learning and memory, and on rheological properties of blood on the background of selective and nonselective inhibition of various nitric oxide synthases (NOS) will not only allow to identify possible disorders of the brain functions, but also pinpoint the physiological mechanisms of these disorders, as well as some unknown aspects of therapeutic or/and damaging effects of WBH. The series of experiments were carried out on the groups of white rats placed in a special hyperthermia chamber (400C) for one hour, without any restrictions in freedom of movement. Multi-way maze was used to study the processes of learning and memory (before and after WBH exposure). Rheological properties of blood (aggregability and deformability of RBC, as well as plasma viscosity) has also been analysed. Analysis received data and data presented in literature allow to make the following conclusions, having not only theoretical, but also practical meaning: 1.WBH might be used as one of the most effective triggering factor for launching of hormetic mechanism, especially in the cancer clinic. 2.The critical is to make sure that chosen dose of WBH (temperature and duration) not exceeds the hormetic range. 3.We believe that in case of WBH an upper limit for used temperature must not exceed 40 degree of Celsius, because inhibition of Hormesis mechanism and unavoidable changes in patient's blood rheology might have a significant negative side effects.
Disclosures: M. Devdariani: None.

Poster

523. Stress: Factors Affecting Sensitivity, Protection, and Recovery

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 523.07/V42

Topic: E.05. Stress and the Brain

Support: Air Force Medical Support Agency

Title: Longitudinal impact of high altitude exposure: from brain imaging to transcriptome

Authors: *N. P. CRAMER1,4, A. KOROTCOV2,5, D. HOLMAN3, A. BOSOMTWI2,5, X. XU1, M. K. JAISWAL1,5, C. TANKERSLEY1, D. P. PERL4,5, S. JONES2,5, B. J. DARDZINSKI2,5, Z. GALDZICKI1,5,
1Anatomy, Physiol. and Genet., 2Dept. of Radiology and Radiological Sci., 3Mol. & Cell Biol. Grad. Program, 4Dept. of Pathology, Uniformed Services Univ. of the Hlth. Sci., Bethesda, MD; 5Ctr. for Neurosci. and Regenerative Med., Bethesda, MD

Abstract: Physical and psychological injuries frequently drive maladaptive changes in brain neuronal and vascular networks. Interactions between environmental and psychological stressors may leave individuals at a greater risk of adverse neuropathological or behavioral disorders such as PTSD. Using a model of chronic hypobaric-hypoxia in the mouse, we can monitor and identify important contributors to maladaptive neuronal and vascular responses by examining the effects of on the transcriptome and brain anatomy following high altitude exposure. Two strains of commonly investigated mice, C57Bl6 and BALBc, are exposed to a simulated high altitude environment of 5000 meters for up to three months. Throughout this period, mice are subjected to behavioral tests as well as neuropathological assessments at intermediate time points. We demonstrated that mice exposed to high altitude show signs of hippocampal dysfunction in contextual fear conditioning tasks and evidence of microglia activation, particularly in white matter tracts. This enhanced inflammatory signature is also present in the transcriptome of hippocampal and amygdala tissue in both strains of mice. Transcripts related to angiogenesis are also significantly upregulated following chronic hypobaric-hypoxia. In vivo imaging of high altitude exposed mice revealed a time-dependent decrease in blood perfusion in the neocortex despite an increase in the vascular density index (VDI). Similarly, MRI derived T2 relaxation times are elevated in the hippocampus and neocortex suggestive of ongoing inflammatory processes consistent with the findings of the transcriptome analysis. We also observe a decrease in apparent radial diffusivity of endogenous tissue water within the corpus callosum suggesting...
inflammation may be driving demyelination under high altitude exposure. In summary, our results suggest that chronic hypobaric-hypoxia causes maladaptive changes resulting in adverse neuropathological and behavioral deficits, accompanied by an abnormal transcriptional signature. These changes may shift the brain to a vulnerable state, which is more susceptible to subsequent stressors, thus leading to a greater risk for adverse outcomes.


Poster

523. Stress: Factors Affecting Sensitivity, Protection, and Recovery

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 523.08/V43

Topic: F.02. Animal Cognition and Behavior

Support: George Mason University OSCAR Grant

Title: Dietary copper reduction and zinc supplementation: An examination of body weight and its mediating effects on fear extinction

Authors: *C. NEELY, S. WILKINS, M. SMITH, J. FLINN; Dept. of Psychology, George Mason Univ., Fairfax, VA

Abstract: Zinc (Zn) and Copper (Cu) are biometals involved with a variety of behavioral abnormalities and neurological disorders. Zn and Cu actively compete with one another during absorption; thus Zn supplementation can result in an indirect Cu deficiency (Nations et al., 2008; Osredkar & Sustar, 2011). A diet with reduced Cu levels administered starting in utero and continuing to four months induced deficits in fear extinction (Neely et al., 2014). An earlier experiment showed that Zn-induced deficits in spatial memory and fear extinction were remediated through Cu supplementation (Railey et al., 2010), suggesting that the effects of excess Zn may be due to a Cu deficiency. In addition, general findings suggest that Cu deficiency has been linked to poor weight gain and ataxia (Everson, Tsai, & Wang, 1967), prompting the need to explore its effects on behavioral measures. The current study utilized a standardized 7012 diet (7012H) formulated with nutritionists at Harlan Laboratories with 23ppm Cu, the 7012H diet with Cu levels reduced to 7-12 ppm (7012Cu), and the 7012H diet with...
10ppm Zn administered through drinking water (7012Zn). We included a “copper control” diet with 16ppm Cu (CC) used in past studies (Howell et al., 2014; Lippi et al., 2014) and compared it to the 7012H diet. Sixty-six Sprague-Dawley rats were administered one of four diets for approximately four months and underwent cued fear conditioning and extinction testing. Weight differences could have mediated the relationship between diet and freezing responses as indicated by a significant mean weight difference (omnibus p<0.01) at the time of testing with our CC group (464g) weighing significantly more than the other groups (7012H =428.46g; 7012Cu=416.55g; 7012Zn=437.46g). As expected, there were no reported differences among diets in conditioning, F(3, 62) = 1.22, p=0.31. On extinction, a significant tone*diet interaction (p=0.02) demonstrated that the CC animals, who weighed the heaviest, exhibited significantly weaker extinction compared to the 7012Cu- and 7012Zn animals. The 7012H animals exhibited intermediate levels of fear extinction. By extinction recall, the 7012H group exhibited similar freezing percentages as the 7012Cu and 7012Zn groups with no significant differences reported. The CC group, which weighed the heaviest, had elevated freezing responses throughout extinction and recall. Open field results will confirm that our results are not confounded by a motor impairment. Extraneous ingredients such as soy, phytates, and high fat content may have impacted our results. Our data emphasize the necessity to examine extraneous variables, such as weight changes and dietary factors.

Disclosures: C. Neely: None. S. Wilkins: None. M. Smith: None. J. Flinn: None.

Poster

523. Stress: Factors Affecting Sensitivity, Protection, and Recovery

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 523.09/V44

Topic: E.05. Stress and the Brain

Support: CONACYT 238313
IMSS 201132

Title: Chronic exposure to environmental noise during puberty, improves the execution of a working memory task and the astrocyte proliferation

Authors: *T. G. MORALES1, G. YAÑEZ-DELGADILLO2, P. HERNANDEZ2, G. CHIPRES-TINAJERO2, R. RAMOS-ZUÑÍGA2, J. ESTRADA-GARCÍA2, S. LUQUÍN2, Y. RUVALCABA-DELGADILLO2;
Abstract: The experiences during development influence brain maturation and might alter the neuroendocrine and cognitive systems. Relevant environmental stimuli may activate the stress response and thus the release of glucocorticoids (GC). This experiences may alter the structure and function of the limbic brain regions that regulate the response to stressful events such as the medial prefrontal cortex (mPFC) and facilitate or impair memory processes. The mPFC participates in cognitive processes such as working memory. Astrocytes are cells that have important functions in the Central Nervous System and they have glucocorticoid receptors. In this study was examined whether exposure to noise stress during development (pre-puberty) could generate effects on working memory and astrocyte population. Male rats Swiss Wistar were exposed to stress environmental noise (EN) during postnatal (PN) 21-36 days. In the present study was evaluated the performance of a working memory task immediately after exposure to EN in T Maze (delay-alternating) and astrocyte numbers in mPFC. Additionally, serum corticosterone levels were measured in these rats. The noise stress significantly increased corticosterone and positively affected the performance of a working memory task particularly at the alternation task with delay. Besides, it was found a difference in the number of astrocytes in the mPFC the group EN. These findings suggest that exposure to environmental noise during development, improves cognitive processes involved in working memory.


Poster

523. Stress: Factors Affecting Sensitivity, Protection, and Recovery

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 523.10/V45

Topic: E.05. Stress and the Brain

Support: School of Life Sciences Undergraduate Research (SOLUR) Program

SOLS Student Innovation Research Challenge Grant

Title: The proteomic profile of chronic stress and recovery in the hippocampus and amygdala
Abstract: The stress response facilitates our ability to deal effectively with threatening situations, but exposure to severe or chronic stressors can lead to a number of undesirable neural, physiological, and behavioral outcomes. Chronic stress is associated with structural changes in the rat hippocampus and amygdala, with corresponding changes in behaviors such as enhanced fear responses, depressive-like behaviors, and some deficits in learning and memory. More recent animal studies have uncovered an inherent neuroplasticity that allows certain brain regions to recover from the stress-induced neural changes. Underlying cellular mechanisms likely involve several different cellular and molecular pathways. In order to gain a more comprehensive understanding of the differences in protein expression throughout the timeline of chronic stress and recovery, chronically stressed and recovered rat hippocampus and amygdala proteomes were sequenced using bottom-up proteomics, consisting of high-performance liquid chromatography (HPLC) and tandem mass spectrometry. Male Sprague-Dawley rats were randomly assigned to chronic restraint stress for 6hr/d/10d or 6hr/d/21d (Str-Imms or Str-Imml), stress for 6hr/d/21d followed by a recovery period of no stress for 10 or 21 days (Str-Recs or Str-Recl), or a control group (Con). We hypothesize that the neural recovery process involves a suite of proteins associated with neuronal plasticity, including synaptic and cytoskeletal proteins and neurotrophins. We optimized sample preparation of brain tissue for mass spectrometry to maximize lipid depletion and minimize protein loss. Our methods discovered over 2,300 proteins in non-stressed hippocampal tissue, including small protein markers of 35 kDa or less such as synaptobrevin, calmodulin, and activity-regulated cytoskeleton-associated protein. We are now investigating hippocampal cytosolic, nuclear, and membrane proteins obtained from post-stress recovery rats. Immunoassay techniques will also be performed in order to determine the quantitative and qualitative changes in candidate protein markers identified by mass spectrometry. Collectively, these results will demonstrate the complexity behind the brain’s mechanisms for responding to chronic stress along different time points and indicate the overall impact of a recovery period. Our integrative approach will also provide a resource for further investigations into the mechanisms of the brain’s recovery from chronic stress.

Title: TrkB mediates the recovery from chronic stress-induced spatial memory deficits and CA3 dendritic retraction

Authors: J. M. ANGLIN, J. B. ORTIZ, P. R. PAODE, S. B. TAYLOR, N. E. MAALOUF, S. KEMMOU, K. NISHIMURA, *C. D. CONRAD;
Psychology, Arizona State Univ., Tempe, AZ

Abstract: Chronic stress leads to hippocampal-mediated spatial learning and memory deficits and hippocampal CA3 dendritic pruning. When chronic stress subsides and a post-stress recovery period ensues, spatial ability and CA3 dendritic complexity recovers to pre-stress levels. Recently, we reported that hippocampal BDNF is necessary for spatial memory improvements during the recovery period after chronic stress ends (Ortiz et al., 2014). However, BDNF was manipulated throughout the chronic stress period and into the post-stress recovery phase. This made it unclear whether hippocampal BDNF was necessary during the recovery period specifically, or whether the outcome reflected delayed effects from the chronic stress period. Since then, a new drug has become available (ANA-12) that can penetrate the blood brain barrier and antagonize the TrkB BDNF receptor. Consequently, we investigated BDNF’s role during the post-stress recovery phase specifically and determined whether the TrkB receptor is necessary for spatial memory improvement during the post-stress recovery period. Male Sprague-Dawley rats were chronically stressed by restraint (6hr/d/21d) and then tested in a cognitive battery soon after restraint ended (Str-Imm) or after a three week post-stress recovery period (Str-Rec). Rats were injected daily with ANA-12 (.5 mg/kg or veh, i.p.) during the post-stress recovery period. Spatial ability was assessed with a radial arm water maze (RAWM) and object placement (OP), and then brains were removed and processed for Golgi staining to assess hippocampal CA3 dendritic complexity. As reported in our other studies, all groups learned the RAWM task similarly, by making fewer errors as trials progressed. Differences manifested on the memory retention trials for both RAWM and the OP paradigm. ANA-12 treatment impaired memory retention of the Str-Rec condition in both the RAWM and OP: Str-Rec-ANA showed poor performance that was similar to Str-Imm-Veh, whereas Str-Rec-Veh performed as well as Con-Veh on both tasks. Moreover, hippocampal CA3 dendritic complexity mirrored the behavioral outcomes: ANA-12 blocked the enhanced dendritic arborization that occurs in the post-stress recovery period (Str-Rec-ANA vs Str-Rec-Veh). These results indicate that during the
post-stress recovery period, TrkB signaling is necessary for hippocampal plasticity, as it pertains to spatial ability and CA3 dendritic complexity.


Poster

523. Stress: Factors Affecting Sensitivity, Protection, and Recovery

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 523.12/V47

Topic: E.05. Stress and the Brain

Support: Arizona State University's College of Liberal Arts and Sciences (Conrad)

Arizona State University's School of Life Sciences Undergraduate Research Program (Daas and Paode)

the National Science Foundation Graduate Research Fellowship Program (DGE-1311230, Ortiz)

Title: Downregulating hippocampal BDNF expression blocks recovery from chronic stress-induced hippocampal CA3 dendritic retraction

Authors: *J. B. ORTIZ†, E. J. DAAS†, A. N. HOFFMAN†, E. FONSECA-TRUJILLO†, P. R. PAODE†, S. KEMMOU†, N. E. MAALOUF†, E. F. TERWILLIGER‡, C. D. CONRAD‡; †Dept. of Psychology, Arizona State Univ., Tempe, AZ; ‡Dept. of Med., Beth Israel Deaconess Med. Center, Harvard Med. Sch., Boston, MA

Abstract: Chronic stress-induced hippocampal CA3 dendritic retraction and deficits in hippocampal-mediated behaviors return to levels of non-stressed controls, i.e. recover, following a post-stress recovery period. We previously demonstrated that hippocampal BDNF is necessary for recovery from spatial memory deficits in the weeks following the end of chronic stress (Ortiz et al., 2014). Here, we investigated whether downregulated hippocampal BDNF blocks the reversal of chronic stress-induced dendritic retraction of hippocampal CA3 neurons following a similar post-stress recovery period. We used RNA interference to downregulate BDNF expression within the dorsal hippocampus. Specifically, viral vectors containing the coding information for an shRNA directed against BDNF (shRNA) or a scrambled sequence (Scr) were infused into the dorsal hippocampus targeting the CA3 region. Young male Sprague-Dawley rats received bilateral hippocampal infusions of shRNA or Scr (3 infusions per hemisphere, 0.2 µl per
Rats were then assigned to chronic stress (6h restraint/d/21d) or unstressed controls (Con). Brains were extracted following a 21-day post-stress recovery period (Str-Rec) or immediately following the end of chronic stress (Str-Imm). Brains were hemisected and one hemisphere was flash frozen for BDNF protein quantification and infusion accuracy assessment, while the other hemisphere was processed for Golgi Stain (FD Rapid GolgiStain Kit). The different hippocampal CA3 neuronal types (short shaft and long shaft) show inherent differences in dendritic complexity; therefore, both neuronal types were equally represented within each rat. The neurons were then traced using a light microscope and a camera lucida drawing tube, with at least eight neurons traced per rat. Dendritic complexity was determined by quantifying the total number of bifurcations and dendritic length. The data show that chronic stress led to a reduction in dendritic complexity: Str-Imm-Scr rats showed fewer dendritic branches compared to Con-Scr. Rats that were allowed a post-stress recovery period and infused with the Scr virus (Str-Rec-Scr) displayed a dendritic profile similar to that of control rats. Importantly, BDNF knockdown appeared to hinder the recovery of CA3 dendritic architecture: rats in the Str-Rec-shRNA group had decreased CA3 dendritic complexity compared to their Str-Rec-Scr counterparts. Together with our previous report, these data suggest that BDNF mediates two forms of recovery in the hippocampus following termination of chronic stress: enhancement of CA3 dendritic arborization and improvement in hippocampal-dependent spatial cognition.


Poster

523. Stress: Factors Affecting Sensitivity, Protection, and Recovery

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 523.13/V48

Topic: E.05. Stress and the Brain

Support: T32 DA07288

DoD W81XWH-11-1-1245 Subaward 803-237

Title: Repetitive oxytocin treatment following traumatic stress blocks stress-induced reinstatement of methamphetamine-seeking and neuroadaptations in the prefrontal cortex and hypothalamus
Abstract: Previous research has shown a strong link between neuropsychiatric disorders such as posttraumatic stress disorder (PTSD) and substance use disorders (SUD). Stimulants, such as methamphetamine (Meth), are among the most highly abused by those with comorbid PTSD who exhibit a longer history of Meth use and poorer overall treatment outcomes than individuals with Meth SUD alone. Moreover, traumatic stress exposure independent of PTSD development can precipitate relapse to abuse in recovering SUD individuals. Negative regulation of the endogenous oxytocin system is thought to increase vulnerability to SUD following traumatic stress exposure. We previously showed that in rats with a history of exposure to the predator odor, 2,4,5-trimethylthiazolin (TMT), a single dose of oxytocin (OXT) when injected systemically 30 mins before a conditioned cue or TMT–induced reinstatement test, was able to suppress reinstatement of Meth seeking to both stimuli in rats with a Meth self-administration (SA) history (Ferland et al submitted). The goal of the present study was to examine whether chronic stimulation of the endogenous oxytocin system following traumatic stress exposure would attenuate Meth-seeking. In rats exposed to TMT or saline for 5 days followed by 10 days of 1.0 mg/kg, i.p. oxytocin (OXT) or saline, we examined (1) TMT (stress)-induced reinstatement of Meth-seeking in rats with a Meth SA history and (2) neuroendocrine alterations and mRNA expression in the dorsomedial prefrontal cortex (dmPFC) and the paraventricular nucleus of the hypothalamus (PVN). TMT pre-exposed (PE) rats injected with saline for 10 days prior to meth SA acquisition exhibited significantly more stress-induced reinstatement of meth-seeking than saline-PE rats injected with either saline. In contrast, repetitive OXT treatment for 10 days suppressed TMT-induced Meth-seeking in TMT-PE and Saline-PE rats. Oxt mRNA in the PVN and OxtR mRNA in both the PVN and dmPFC were significantly less in TMT-PE rats injected with saline than in saline-PE controls. Ten days of OXT prior to acquisition of Meth SA prevented the decrease in Oxt and OxtR mRNA in TMT-PE rats. In addition, TMT-PE rats treated with saline showed a significant reduction in the epigenetic mark Hdac5 and a corresponding increase in Bdnf mRNA in dmPFC compared to saline PE animals. Repetitive OXT attenuated the Hdac5 decrease and normalized Bdnf mRNA in the dmPFC of TMT PE rats. The results from these studies support oxytocin as a novel therapeutic strategy for SUD with concurrent PTSD. Supported by T32 DA07288 and DoD W81XWH-11-1-1245 Subaward 803-237

Disclosures: C.L. Ferland: None. E.L. Herzig: None. J.F. McGinty: None.
The effects of the TrkB agonist, 7,8-dihydroxyflavone, on rats’ stress response to predator odor

Authors: *J. KOERBER, C. L. FERLAND, T. S. DENNIS, S. M. BARRY, E. L. HERZIG, J. F. MCGINTY; Neurosci., Med. Univ. of South Carolina, Charleston, SC

Abstract: Brain derived neurotrophic factor (BDNF) promotes neuronal survival and modulates the rodent stress response through activation of its high affinity tyrosine receptor kinase, TrkB. Although BDNF has tremendous preclinical value, it is hindered by its limited ability to cross the blood-brain barrier. In contrast, the selective TrkB agonist 7,8-dihydroxyflavone (7,8-DHF), readily crosses the blood-brain barrier and has therapeutic effects in several rodent studies modeling stress and learning. Furthermore, our lab has previously demonstrated that rodents repeatedly exposed to 2,4,5-trimethylthiazoline (TMT), a fox fecal extract, have an increased level of peripheral corticosterone, exhibit anxiety-like behavior in behavioral tests, and show augmented reinstatement of methamphetamine-seeking that persists weeks after the initial predator odor exposure. These findings suggest that TMT exposure is an efficacious animal model of posttraumatic stress disorder (PTSD). Based on these findings and others suggesting BDNF is a modulator of stress, we decided to investigate 7,8-DHF’s effect on the rodent stress response. We exposed rats to a filter paper soaked in TMT (10 ul, 1% solution, 15 min. sessions) or saline (10 ul, 15 min. sessions) once daily for five days in open field boxes, after three days of pre-exposure habituation. Two hours before the trials, we administered either 7,8-DHF (5 mg/kg, i.p. dissolved in 17% DMSO) or vehicle (17% DMSO) to investigate whether 7,8-DHF would attenuate any component of the stress response induced by exposure to the predator odor. Immediately following the fifth day of TMT exposure, rats were rapidly decapitated without anesthesia, their brains were extracted, and tissue collected from the dorsomedial prefrontal cortex (dmPFC), amygdala (AMY), paraventricular nucleus (PVN), and hippocampus (HIP). Additionally, adrenal glands were weighed and trunk blood was collected for corticosterone analysis. Preliminary results indicate no difference in adrenal weights between groups but the rats exposed to TMT all gained significantly less weight during the five days of exposure and exhibited elevated corticosterone levels, indicating a deleterious response to traumatic stress. However, 7,8-DHF did not affect these measurements. Protein and mRNA levels will be analyzed using the WES system (Protein Simple) and qPCR, respectively. It is expected that the TMT-exposed rats will have reduced levels of bdnf mRNA and reduced levels of activated TrkB,
which will be prevented by 7,8-DHF. Acknowledgements: Supported by DA033479 and GAANN


Poster

523. Stress: Factors Affecting Sensitivity, Protection, and Recovery

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 523.15/W2

Topic: E.05. Stress and the Brain

Title: EEG-mediated perceived stress reduction through guided subtraction meditation

Authors: *G. Z. SOGHOYAN*1,2, G. K. SCHEINER2, R. J. GOUGELET2, A. M. VAHID3, J. A. PINEDA2,4;

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Abstract: Unique methods that effectively reduce, prevent, and even temporarily provide relief from stress are of utmost importance to translational neuroscience research. Not only can these methods have immediate application to those suffering, but they can provide insights into cognitive behaviors that lead to unhealthy levels of stress. We investigated the efficacy of Guided Subtraction Meditation (GSM), a new meditation practice from South Korea that claims to have a method that quickly allows practitioners to dissociate from past traumatic experiences, eliminate stressful patterns of thinking and reduce the occurrence of negative affective and physiologically hyperaroused states. GSM’s method involves detaching from life experiences stored in the mind as thoughts and images of the past, present, and future by bringing them up systematically and “letting them go.” In a relatively short period (100-150 hrs of practice), this method claims to be able to lead practitioners into a state of stress free, present, and non-judgemental awareness. To determine if this practice actually has the beneficial impacts related to stress and cognition that it claims, we longitudinally tracked practitioners of this meditation (n=15) alongside non-meditating control subjects (n=20) from their first hour of meditation practice to their 300-350th hour of meditation practice. At three timepoints (start, 100-150 hrs and 300-350 hrs of practice or every 8-14 weeks for non-meditator group), we administered Cohen’s Perceived Stress Scale and neurophenomenological questions pertaining to stress linked cognitive activity. We also recorded brain EEG activity under three conditions: an eyes closed baseline, a session of GSM, and a “stillness” task, during which time subjects were asked to refrain from thinking. Between start and mid- treatment timepoints, we found trending decreases
in trait cognitive stress, which co-occurred with changes in EEG activity consistent with past findings. We found that across all conditions, there were increases in both anterior right-lateralized 10Hz Alpha and in Frontal Midline 7Hz Theta (FMθ) EEG power. Lateralized Alpha changes indicate there was an increase in differential PFC activity, implicated in the processing of positive and negative affective states. The FMθ increases indicate decreased DMN activity associated with stress related mental function. These results suggest that GSM produces significant stress reduction in a normal healthy population, which has implications for use in treating stress related illnesses such as anxiety, depression, and cardiovascular diseases.


Poster

523. Stress: Factors Affecting Sensitivity, Protection, and Recovery

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 523.16/W3

Topic: F.02. Animal Cognition and Behavior

Support: NIH training grant T32NS082145

NIMH research grant R01MH072672

William and Ella Owens Medical Research Foundation grant

Title: Modeling cognitive therapy in the rat: plasticity associated with fear extinction may underly reversal of chronic stress-induced behavioral deficits

Authors: *E. A. FUCICH¹,², D. A. MORILAK¹,²;
¹Pharmacol., ²Ctr. for Biomed. Neurosci., Univ. of Texas Hlth. Sci. Ctr., San Antonio, TX

Abstract: Stress-related psychiatric disorders, like depression or post-traumatic stress disorder (PTSD), are prevalent yet poorly treated. These disorders share cognitive flexibility deficits associated with medial prefrontal cortex (mPFC) dysfunction. Psychotherapies invoking cognitive flexibility can be efficacious even in pharmacotherapy-resistant patients, although, as with pharmacotherapies, response to psychotherapy can be incomplete, some patients do not respond, and relapse remains an issue. Thus, understanding the neurobiological mechanisms underlying its efficacy could inform more rapid, efficacious, or long-lasting behavioral therapies, or could inform the development of adjunct treatment strategies designed to improve its effect. Pre-clinically, we have shown that chronic unpredictable stress (CUS) causes deficits in mPFC-
mediated cognitive flexibility in the attentional set-shifting test (AST), and induces maladaptive coping behavior in the shock-probe defensive burying (SPDB) test in rats. We have shown that fear extinction learning, which engages mPFC cognitive flexibility and conceptually resembles prolonged exposure therapy for PTSD in humans, can model cognitive therapy in rats by improving performance in the AST and SPDB tests that have been compromised by chronic stress (SfN Abstract 468.07, 2014). This study tested whether extinction induced protein translation and phosphorylation of plasticity-related synaptic proteins in the mPFC of stressed rats whose cognitive and behavioral coping deficits are reversed by extinction therapy. Western blot analyses of mPFC tissue showed elevated phosphorylation of ribosomal unit S6 (pS6) in stressed rats after extinction compared to baseline. Similar results were found in the lateral septum, a sub-cortical target of mPFC that mediates defensive behavior on the SPDB test. Chronic stress increased phosphorylation of AMPA receptor subunit GluR1 at site S831 in the mPFC, and extinction treatment normalized pGluR1(S831) to non-stress baseline levels 24 hr after treatment. These results suggest that fear extinction induces protein translation and changes in the phosphorylation of plasticity-related proteins in the mPFC and at least one of its downstream targets. Such processes may be important to the beneficial effects on cognition and coping behaviors that have been compromised by chronic stress, and may suggest targets for the development of adjunct pharmacological tools to enhance the efficacy of behavioral therapy.

**Disclosures:** E.A. Fucich: None. D.A. Morilak: 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.; Lundbeck A/S. F. Consulting Fees (e.g., advisory boards); Lundbeck A/S.

**Poster**

523. Stress: Factors Affecting Sensitivity, Protection, and Recovery

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#Poster#:** 523.17/W4

**Topic:** E.05. Stress and the Brain

**Support:** 1ZIAMH002784

**Title:** Do new neurons in the hippocampus buffer long-lasting effects of traumatic stress on anxiety-like behavior and learning?

**Authors:** *T. J. SCHOENFELD, H. A. CAMERON;
NIMH/NIH, Bethesda, MD
Abstract: New neurons in the hippocampus are functionally important for normal regulation of the hypothalamic-pituitary-adrenal (HPA) axis response to acute stress, and animals without adult neurogenesis are more susceptible to behavioral abnormalities following mild acute stress. The hippocampus has been implicated in post-traumatic stress disorder (PTSD), with hippocampal volume loss often found with patients suffering from PTSD. The hippocampus is known to be highly plastic in response to stress, and adult neurogenesis in the dentate gyrus is highly regulated by stressful experiences. We tested whether new neurons in the hippocampus were functionally important for behavioral changes associated with traumatic stress. We used valganciclovir to inhibit adult neurogenesis in GFAP-TK (TK) transgenic rats for 8 weeks. Both TK rats and wild type (WT) littermate controls underwent a single-prolonged stress (SPS) procedure, which has been shown to have delayed and long-lasting effects on learning, memory, and affect, and is thought of as an animal model for PTSD. 1-3 weeks following SPS, WT and TK rats were tested on a variety of behavioral paradigms reflecting anxiety-like behavior (novelty-suppressed feeding), spatial memory (object location test), and fear learning (contextual fear conditioning and extinction). SPS reduced proliferation and adult neurogenesis 1 week following stress in WT rats. SPS was anxiogenic in WTs but had a stronger anxiogenic effect in TKs. SPS altered the behavior of WT rats in an object location task; SPS had no effect on behavior in TKs, but both control and stressed TKs behaved differently than WT controls. SPS increased contextual fear conditioning in TKs but had no effect on WT controls. The results suggest that rats without new neurons in the hippocampus may be more susceptible to the effects of traumatic stress on behavior.

Disclosures: T.J. Schoenfeld: None. H.A. Cameron: None.

Poster

523. Stress: Factors Affecting Sensitivity, Protection, and Recovery

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 523.18/W5

Topic: E.05. Stress and the Brain

Support: Public Health Research Foundation

Title: Daily amount of spontaneous running volume influences stress responses and corticotrophin-releasing hormone levels

Authors: *S. YANAGITA, N. KUBOTA, Y. TAKANO, K. TAKEDA; Tokyo Univ. of Sci., Chiba, Japan
Abstract: Regular physical exercise can relieve stress response and increase stress tolerance. In animal studies, it has revealed that spontaneous running using running wheels is particularly effective to enhance anti-stress action. Daily amount of physical exercise volume is an important factor to obtain beneficial effects of physical exercise, although our previous study showed that there are considerable individual differences on the daily amount of spontaneous running distance in rats. It is thus possible that daily exercise volume may influence stress relieving effects. In this study, we focused on the effects of daily amount of spontaneous running on neuroendocrine response to several kinds of stress in rats. Male Wistar rats were housed individually in cages with or without an attached running wheel. Physically active rats were allowed voluntary access to their wheels for 4 weeks. The rats were screened into high runner or low runner based on the calculated daily running distance. Following to 4 weeks running sessions, the rats in both high and low runners were received severe (foot shock) or mild (psychological) stress conditions. We assessed the levels of brain corticotrophin releasing hormone (CRH), and plasma ACTH and corticosterone using ELISA following to stress stimulations. We also calculated the body and adrenal weights as an index of chronic stress response. The results showed that CRH levels in the several brain regions following to severe stress stimulation were not different between high and low runner although CRH levels in both high and low runners received stress were lower than that in control rats. Plasma ACTH levels after receiving stress in runners was higher than controls. These results suggest that daily physical exercise indeed induces anti-stress action, but these reactions are independent of amount of physical exercise in the case of severe stress conditions. On the other hands, mild psychological stress via contextual fear conditioning test induced different reactions between high and low runners on changing levels of brain CRH following to stress stimulation. This result leads to the hypothesis that severe stress stimulation could buffer the anti-stress action of daily physical exercise, suggesting that daily amount of physical exercise volume may influence stress tolerance via physical exercise to a certain degree. These results of present study suggest that it is require investigating the detail of relationship between exercise conditions and anti-stress action.

Disclosures: S. Yanagita: None. N. Kubota: None. Y. Takano: None. K. Takeda: None.

Poster

523. Stress: Factors Affecting Sensitivity, Protection, and Recovery

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 523.19/W6

Topic: E.05. Stress and the Brain
Support: NIH Grant R15 MH104485
NIH Grant P20 RR15567
CBBRe Research Enhancement Pilot grant
an anonymous donor to the Summers’ lab via the USD Foundation

Title: Exercise modifies orexin receptor, BDNF, and TrKB receptor gene expression during social stress: Ventral versus dorsal hippocampal regions

Authors: *T. R. SUMMERS¹,², J. K. ACHUA¹,², J. P. SMITH¹,²,³,⁴, M. A. PRINCE¹,², J. M. ROBERTSON¹,², C. H. SUMMERS¹,²;
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Abstract: Hippocampal roles in anxiety, stress, emotion, fear conditioning, and depression may be regionally distinctive. The dorsal hippocampus is thought to be primarily responsible for spatial memory and learning, while ventral hippocampus is associated with anxiety, emotion, fear conditioning, and stress. We hypothesized that repeated social defeat using the Stress Alternatives Model (SAM) would identify differences in gene expression for Orx₁ and Orx₂ receptors as well as BDNF and its TrK_B receptor in the dorsal and ventral hippocampus. We further hypothesized that changes in gene expression, measured in dentate gyrus (DG) and CA₁, would correlate regionally with anxious behavior. The SAM arena is an oval open field with portals only large enough for smaller C57BL6/N mice, allowing for escape when mice are subject to attacks by a larger novel aggressive CD1 mouse over four daily (5 min) trials. In the SAM, social interactions result in an even split of escape or submission, with stable behavioral phenotypes established by the second day of interaction. To test for the potential anxiolytic properties of exercise, some mice had access to a running wheel for the duration of the experiment. Stress-induced changes in gene expression for orexin receptors were more prevalent in ventral hippocampus. In the absence of exercise, anxiogenic activity (aggressive social interaction in SAM) diminished Orx₁ and Orx₂ gene expression in the ventral CA₁ of submissive mice, but also decreased Orx₁ mRNA in escaping animals. Decreased ventral CA₁ Orx₁ and Orx₂ gene expression was similar in exercising mice for both escaping and submissive mice. Additionally, both escaping and submissive mice with running wheel access had decreased Orx₂ mRNA in the ventral DG. The only effect found in dorsal hippocampus was in exercising mice, where Orx₂ mRNA in the DG increased in both escaping and submissive mice. In dorsal CA₁, social submission stress reduced BDNF mRNA, which was reversed and increased by voluntary exercise. Social submission also decreased BDNF gene expression in ventral DG, which was alleviated by exercise. Social stress increased TrK_B gene expression in ventral DG for both escaping and submissive animals, which was also reversed and diminished by exercise. The results suggest that social stress-induced changes in gene expression of orexin receptors, BDNF,
and TrK receptors are more prevalent in the ventral hippocampus, but both dorsal and ventral regions appear to be involved. We suggest that Orx₁, but especially Orx₂ receptors of the ventral hippocampus are involved in mediation of anxious and depressive behaviors related to social stress.


Poster

523. Stress: Factors Affecting Sensitivity, Protection, and Recovery

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 523.20/W7

Topic: E.05. Stress and the Brain

Support: PSC-CUNY Research Award (TRADA-45-619)

Center for Translational and Basic Research (CTBR) at Hunter College

BP-ENDURE NIH-NINDS Grant # R25NS080686

Title: Exploring the effects of curcumin on morphological changes in the lateral amygdala following chronic corticosterone exposure in rats

Authors: *H. KHANDAKER¹, M. A. BRIONES¹, M. SELIGSOHN², T. WINER², S. CHIN², K. LOPEZ², G. SCHAFE¹²;

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Abstract: Chronic stress has been strongly implicated in the development of a number of psychiatric disorders, including post-traumatic stress disorder (PTSD). The release of glucocorticoid stress hormones in response to chronic stress has been found to have contrasting effects on both morphology and physiology within different regions of the brain enriched with glucocorticoid receptors such as the amygdala, hippocampus, and prefrontal cortex. Recent findings in our lab have shown that chronic oral exposure to corticosterone (CORT), a stress-associated adrenal steroid, persistently enhances the expression of the synaptically-localized proteins GluR1 and synaptophysin within the lateral nucleus of the amygdala (LA; Monsey et al, 2014), an effect which is consistent with studies that have observed long-lasting dendritic hypertrophy and increased spine density in LA neurons in chronically stressed rats (Vyas et al, 2004; Mitra et al, 2005). Further, we have observed that these CORT-induced synaptic effects in
the LA are prevented if rats are fed a diet enriched with the polyphenol compound curcumin (derived from the rhizome of Curcuma longa) during the CORT exposure period. In the present study, we directly examine whether chronic CORT exposure is associated with spine changes in the LA, and whether a diet enriched with curcumin may prevent these morphological changes. Male Sprague Dawley rats received chronic exposure to either water or CORT (50µg/ml) in their drinking water for 14 days during the 2-week CORT exposure period, half of the rats received a diet of standard rodent chow, while the other half received chow that was fortified with 1.5% curcumin to comprise 4 groups: 1) Water-regular chow, 2) Water-curcumin chow, 3) CORT-regular chow, 4) CORT-curcumin chow. At the end of the 14-day period, brains were collected and processed using a rapid Golgi staining procedure for spine analysis. Our preliminary findings suggest that 2 weeks of oral CORT exposure increases spine density in the LA, and that a diet enriched with curcumin may prevent this effect. Our findings add further support to the hypothesis that a diet enriched with curcumin may mitigate stress-related changes in the brain.


Poster 523. Stress: Factors Affecting Sensitivity, Protection, and Recovery

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 523.21/W8

Topic: F.02. Animal Cognition and Behavior

Support: Center for Translational and Basic Research (CTBR) at Hunter College

Title: Dietary curcumin enhances extinction of a pavlovian fear memory

Authors: *M. A. BRIONES1, H. KHANDAKER1, M. SELIGSOHN2, A. SEENAUTH2, M. HUSSAIN2, G. SCHAFE1,2;
1Psychology, The Grad. Ctr. CUNY, New York, NY; 2Psychology, Hunter College, CUNY, New York, NY

Abstract: Curcumin, a yellow-pigment compound found in the popular Indian spice turmeric (Curcuma longa), has been extensively investigated for its anti-inflammatory, neuroprotective, and chemopreventative properties. Recently, we have shown that a dietary source of curcumin significantly impairs the consolidation and reconsolidation of Pavlovian fear conditioning, a widely studied animal model of traumatic memory formation in post-traumatic stress disorder (PTSD). Here, we examined the effect of dietary curcumin on the extinction of a Pavlovian fear
memory. Rats were first trained with 3 pairings of a 5 kHz, 75 dB, 30 sec tone conditioned stimulus (CS) that co-terminated with a 1 sec, 0.75 mA footshock (unconditioned stimulus; US). Rats were then fed a diet consisting of either control chow or a 1.5% curcumin-enriched chow for 5 days prior to extinction training consisting of 40 tone-alone CS presentations. Rats fed a diet enriched with curcumin exhibited a significantly faster rate of extinction during training relative to the regular chow control group. Further, when tested for retention of the extinction memory 24 hours later, rats fed the curcumin-enriched diet exhibited significantly enhanced extinction retention relative to controls. Collectively, our findings indicate that dietary curcumin is capable of facilitating extinction learning and enhancing extinction retention. Our findings add further support to the hypothesis that curcumin may be useful as an adjunct in the treatment of psychological disorders such as PTSD that are characterized by fearful memories.

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**Poster**

**523. Stress: Factors Affecting Sensitivity, Protection, and Recovery**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 523.22/W9

**Topic:** E.05. Stress and the Brain

**Support:** NIMH Grant 1R15H101698-01A1

**Title:** Coping strategies influence emotional resilience and neurobiological markers of depressive symptoms in male and female rats

**Authors:** M. KENT¹, M. BARDI¹, A. HAZELGROVE¹, K. SEWELL¹, E. KIRK¹, B. THOMPSON¹, K. TREXLER², B. TERHUNE-COTTER¹, S. SCOTT¹, *K. G. LAMBERT²,¹; ¹Psychology and Behavioral Neurosci., ²Psychology, Randolph Macon Col., Ashland, VA

**Abstract:** Using a rodent model, our laboratory has identified specific coping strategies associated with enhanced resilience against the emergence of depressive symptoms. Rats with a flexible coping phenotype, for example, exhibit increased neurobiological markers of emotional regulation compared to active and passive copers (Bardi et al., 2012; Lambert et al., 2014). In the current study, responses of male and female rats to prediction errors in a spatial foraging task (dry land maze; DLM) were examined after animals were exposed to a depressogenic chronic unpredictable stress protocol for two weeks. Brains were processed following the DLM training/assessment for fos-activation patterns and several measures of neuroplasticity (e.g.,
BDNF; GFAP, ki-67, doublecortin) in the hippocampus and relevant areas. Results indicated that males exhibited more active exploration in the DLM probe test whereas flexible and active females exhibited more strategic rear responses (targeted to interior of maze) in the absence of the predicted reward than their male counterparts. Regardless of sex, a trend for flexible copers to spend more time in proximity to the previously baited well was observed. In a conditioned fear stimulus test, males exhibited more general exploration in response to the fear-stimulus whereas females exhibited increased rear responses and interrupted grooming sequences. Fecal samples collected during baseline and following forced swim exposure revealed higher corticosterone (CORT) in active copers, whereas flexible copers had higher dehydroepiandrosterone (DHEA) and DHEA/CORT ratios, both indications of enhanced emotional regulation. In the DLM probe test assessing the rats response to uncertainty, flexible copers exhibited fewer fos-immunoreactive cells in the basolateral amygdala and a trend toward lower activation in the insula, with no differences observed in the hippocampus and pyriform cortex. Following DLM assessment, coping profiles differentially influenced markers of neuroplasticity (e.g., GFAP and BDNF); additional analyses are currently ongoing as more neuroplasticity markers are being quantified. In sum, despite increased rates of depression reported in human females, sex effects weren’t as pervasive as coping strategies in the neurobiological markers of depressive symptoms and emotional regulation/resilience investigated in the current study. This work was supported by NIMH award 1R15H101698-01A1 to KGL.


Poster
523. Stress: Factors Affecting Sensitivity, Protection, and Recovery
Location: Hall A
Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM
Program#/Poster#: 523.23/W10
Topic: E.05. Stress and the Brain
Support: FAPESP- Brazil CNPq
Title: Chronic Ouabain counteracted the effects of CUS in the HPA axis and CREB signaling
Abstract: Ouabain (OUA), a potent inhibitor of the Na,K-ATPase pump, was identified as an endogenous hormone produced in hypothalamus and adrenal gland. This cardiac glycoside binds to Na,K-ATPase and it can activate signaling pathways in concentrations that is not linked to the common effect of the pump inhibition. It has been demonstrated the involvement of the OUA in the acute stress response, where physical exercise is capable of increasing OUA levels in rats, dogs and humans minutes after the onset of physical activity. The central effectors of the stress response are the corticotrophin releasing hormone (CRH), which stimulates the secretion of adrenocorticotropic hormone (ACTH), and this one acts on the adrenal cortex release glucocorticoid (GC) hormones. GCs in turn act back on the hypothalamus and pituitary in a negative feedback cycle to suppress CRH and ACTH production. Chronic unpredictable stress (CUS) activation has been shown to affect health, making the individual more susceptible to infections, tumors, hypertension, heart attack, stroke, autoimmunity and psychopathology. Furthermore, animal models of CUS memory impairment associated with atrophy of neuron in hippocampus and frontal cortex showed down-regulation BDNF expression. The present work investigated the effects of OUA on CUS induced changes in HPA axis and cyclic AMP response element-binding protein (CREB) expression in the hippocampus, frontal cortex and hypothalamus. Adult male rats were subjected to CUS protocol for 14 days and pre-treated intraperitoneally (i.p.) with Ouabain (1.8 mg/kg) (every other day). Serum CORT (corticosterone, the principal form of GCs in rodents) and ACTH were measured using ELISA kit. Electrophoretic mobility shift assay (EMSA) was used to evaluate CREB activity in brain tissues. Our results showed that CUS induced an increase in serum CORT and ACTH levels and chronic treatment with OUA (1.8mg/kg) counteracted the CUS effect by reducing both CORT (39%) and ACTH (53%). In addition, we found that CUS reduced CREB activity and OUA reverted it only in the pre-frontal cortex. Taken together our results indicate that OUA modulates HPA axis and CREB activities in the pre-frontal cortex. Further studies are necessary for elucidation a putative physiological role of this hormone in chronic stress response.

Disclosures: J.A. Leite; None. A. Orellana; None. P. Kinoshita; None. L. de Sá Lima; None. D. Andreoti; None. E. Kawamoto; None. C. Munhoz; None. C. Scavone; None.
Title: Attenuating epigenetic alterations following exposure to caregiver maltreatment: A role for HDAC inhibition?

Authors: *T. S. DOHERTY, A. K. OHARA, T. L. ROTH; Univ. of Delaware, Newark, DE

Abstract: Quality of maternal care plays a major role in developmental processes and, when disrupted, in the occurrence of aberrant behavioral trajectories. The underlying mechanisms of this relationship remain elusive, though epigenetic processes such as DNA methylation are promising candidates. Previously we assessed alterations in DNA methylation in adolescent rats that had been exposed to our caregiver maltreatment paradigm and discovered that females exhibited gene-specific (bcdnf) changes in response to maltreatment whereas maltreated males exhibited changes on a global level, including global alterations in a recently identified epigenetic modification that plays a role in learning and memory: hydroxymethylcytosine. The aim of the current study was to assess behavioral trajectories (specifically recall of fear memories) resulting from these alterations as well as the effects of an HDAC inhibitor (sodium butyrate, administered concurrently with maltreatment) on preventing these alterations. Infant male and female Long Evans rats were subjected to either nurturing care (from their biological mother or foster dam) or maltreatment from a foster dam for 30 minutes daily from postnatal day (PN) 1 to PN7. Preliminary results indicate that maltreated males exhibit fear extinction retention deficits when compared to controls. Results will be discussed in the framework of consequences, mechanisms, and interventions in early-life stress.

Support: NIMH grant MH050479

Title: Prior injection of ketamine produces an enduring blockade of the effects of an uncontrollable stress

Authors: S. TILDEN¹, K. BARTHOLOMAY¹, K. SPERR¹, N. CIANCIO¹, J. AMAT¹, *S. F. MAIER², L. WATKINS¹;
¹Univ. of Colorado, Boulder, CO; ²Univ. Colorado, BOULDER, CO

Abstract: Inescapable, uncontrollable tail shocks (IS) produce neurochemicals (“learned helplessness”) that include increased anxiety that is dependent on activation of 5-HT DRN neurons that project to the basolateral amygdala (BLA). There is recent interest in antidepressant and anti-stress effects of sub anesthetic doses of ketamine, an NMDA receptor antagonist. We have previously found that ketamine administered ip 2 hrs before inescapable/uncontrollable tail shocks (IS) blunts the increased anxiety and BLA 5-HT typically produced by IS. We also showed that this effect of ketamine appears to depend on the prelimbic region of the medial prefrontal cortex (SFN 2014). Here we report that the blunting of the IS effects by ip ketamine can last up to 2 weeks. A group of Sprague Dawley rats were implanted with a microdialysis cannula guide directed to the basolateral amygdala. They received an ip injection of ketamine (10 mg/Kg) or saline, a week after surgery. 2 weeks after the ip injection they received a session of IS (100 tail shocks) and BLA microdialysis. Another group of rats were injected ip with ketamine (10 mg/Kg) or saline and 2 weeks later received a similar IS session. A juvenile social exploration test occurred 24 hrs later to evaluate the level of anxiety. Ketamine given 2 weeks before IS appears to blunt the serotonergic increase and heightened anxiety produced by IS. Supported by MH050479,

Disclosures: S. Tilden: A. Employment/Salary (full or part-time); University of Colorado at Boulder. K. Bartholomay: None. K. Sperr: None. N. Ciancio: None. J. Amat: A. Employment/Salary (full or part-time); University of Colorado at Boulder. S.F. Maier: A. Employment/Salary (full or part-time); University of Colorado at Boulder. L. Watkins: A. Employment/Salary (full or part-time); University of Colorado at Boulder.

Poster

523. Stress: Factors Affecting Sensitivity, Protection, and Recovery

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 523.26/W13

Topic: E.05. Stress and the Brain
Title: Protective effect of s-allyl cysteine in a chronic restraint model in rats

Authors: *A. L. COLÍN GONZÁLEZ, H. BECERRIL-CHAVÉZ, A. SANTAMARIA;
Inst. Nacional De Neurologia Y Neurocirugia, Mexico, D.F., Mexico

Abstract: How the Central Nervous System responds to chronic stress remains unknown, albeit some studies demonstrate that these responses include neurochemical and morphological and behavioral alterations. An often seen component of chronic stress is oxidative damage, a deleterious event produced by an imbalance between reactive oxygen species (ROS) formation and endogenous antioxidant systems. S-allylcysteine (SAC), a garlic-derived compound, a well-characterized antioxidant, has been shown to scavenge ROS, augment the levels of endogenous antioxidants, and increase the activation of Nrf2. Therefore, SAC is a promising tool for experimental protocols involving oxidative stress. In this work, we tested the effects of SAC on the behavioral, morphological and biochemical alterations in the brain of rats submitted to chronic restraint stress. Rats were immobilized for 6 h per day during 21 days, whereas SAC (50 mg/kg, i.p.) was administered daily as a pretreatment 30 min before the restraint session. At the end of the restraint protocol, different markers of toxicity were estimated: 1) brain morphology, 2) cell death ratio, 3) behavioral tasks (forced swimming test, motor alterations and elevated plus maze), 4) body weight, 5) antioxidant enzymes activity (glutathione peroxidase (Gpx) and glutathione S-transferase (GST)), and 6) lipid peroxidation. SAC reduced the morphological alterations (cell death) in CA3 (hippocampus), striatum and cortex. In addition, the enhanced number of visits that rats under stress made to closed arms in the elevated plus maze -a current index of anxiety- was significantly reduced by SAC. Enzyme activities and lipid peroxidation were both increased in stressed rats in cortex, and subsequently reduced by SAC. These results support a protective role of SAC in the noxious events produced by chronic restrain stress probably exerted by its antioxidant properties.


Poster

523. Stress: Factors Affecting Sensitivity, Protection, and Recovery

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 523.27/W14

Topic: E.05. Stress and the Brain

Title: Stress, mindfulness meditation and theta/beta ratio: a study on EEG oscillation
Authors: *S. YUAN, R. M. ATCHLEY, S. D. GARRETT-RUFFIN, H. C. CROMWELL; Psychology Dept., Bowling Green, OH

Abstract: Stress has been found to alter neural oscillations including theta and beta rhythms. Stress effects upon neural oscillations can be significant and have been found to be related to alteration in emotional regulation and cognition. Examining the ratio between faster (beta) and slower (theta) oscillations could provide more insights into these effects. Our goal of the present study was to explore how stress could alter theta/beta ratios and how this could compare to effects on the individual rhythms. Another goal of the study was to explore how mindfulness meditation practice could impact these stress effects. Mindfulness is a useful tool in clinical work and has been found to reduce stress effects. Mindfulness also has significant effects on brain signals including electroencephalographic recordings, neuroimaging and neurochemical activity. In order to explore the impact of mindfulness meditation in buffering stress and reducing the impact stress has on theta/beta ratio alterations, we trained individuals on mindful meditation prior to stress exposure. The cold-pressor task was used to induce stress. A control group practiced relaxation techniques instead of mindful meditation training. Our results revealed an impact of stress on theta/beta ratio. Stress led to a significantly higher theta/beta ratio compared to pre-stress. The effects of stress on T/B ratio changed for each group. For the most part, mindfulness did not significantly buffer the effects of stress. T/B ratios did seem to be sensitive to each treatment but also changed in the control group over time. This result suggests that T/B ratio may not a stable trait measure. The results also support the view that T/B ratio could be a sensitive marker for the effects of stress and adaptation to stress over time. One avenue for future work is to explore how theta/beta ratio could be used to predict stress effects and the effectiveness of treatment for emotional dysregulation.


Poster

523. Stress: Factors Affecting Sensitivity, Protection, and Recovery

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 523.28/W15

Topic: E.05. Stress and the Brain

Title: Effect of Aged Garlic Extract on mRNA levels of GLUT1, GLUT3 and GLUT4 in brain of diabetic rats
Abstract: Glucose is the main source of energy for mammalian brain, glucose is internalized by glucose transporter proteins (GLUT) in cells. In brain are abundantly expressed GLUT1, GLUT3, (insulin not dependent) and less proportion GLUT4 (insulin dependent). During diabetes increases the blood glucose levels and brain. This increase causes a high production of reactive oxygen species (ROS), resulting in oxidative stress state, which is an important factor for diabetic complications. ROS are involved in pathways that regulate the expression of the GLUTs, since they are able to activate and inactivate transcription factors involved in gene expression of GLUTs. In recent years it has studied the effect of exogenous antioxidants which regulate oxidative stress, disturbed blood glucose levels and improve diabetic complications.

Aged Garlic Extract (AGE) is an exogenous antioxidant that lowers ROS and glucose levels in animal models, but it has not been studied the effect of AGE on the regulation of the GLUTs in the brain. To investigate it, we use Wistar rats, diabetes was induced with streptozotocin (60 mg/kg). AGE treatment (360 mg/kg) was administered daily for 42 days, at the end of treatment, the glucose was measured and animals were sacrificed, brains were dissected and total RNA extraction, RT-PCR and Real Time PCR was realized. In this model, the glucose levels increased in diabetic group. Treatment with AGE had an anti-hyperglycemic effect. In diabetic group, the GLUT1 and GLUT4 level mRNA tends to decrease without significance changes, whereas GLUT3 have small increase no significant (p>0.05). Administration of AGE caused decreases not significant of GLUT1 (p >0.05), GLUT3 showed slight increase not significant (p>0.05) in the other hand we did find an increased statistically significant of GLUT4 mRNA (p<0.05). It have been showed that oxidative stress could affect DNA binding of transcription regulating factors in hyperglycemia, as the hypoxia inducible factor (HIF) and nuclear factor 1 (NF1). The treatment with AGE, neutralize ROS and HIF could be regulated, this factor active the expression of GLUT1. The expression of GLUT4 is increased by NF1, but during oxidative stress this factor is oxidized, therefore levels decrease GLUT4, the AGE probably induced the increase of NF1, by neutralized ROS. The treatment with AGE is not affect expression of GLUT3 mRNA, we propose that more studies are necessary to elucidate the role of AGE on transcription factors that regulate the expression of GLUT3


Poster

523. Stress: Factors Affecting Sensitivity, Protection, and Recovery
Title: Effects of early cortisol exposure on the developing GnRH neuron system in zebrafish

Authors: D. SKINNER\textsuperscript{1}, S. RAMAKRISHNAN\textsuperscript{2};\textsuperscript{1}Dept of Biology, Neurosci. Program, \textsuperscript{2}Neurosci. Program, Univ. of Puget Sound, Tacoma, WA

Abstract: We investigate the effects of chronic stress on the development of GnRH neurons in the early zebrafish (Danio rerio) embryo. Embryonic exposure to stress has been shown to cause problems in the development of GnRH neurons in other fish species most notably salmon and carp. Exposure to stress in zebrafish has been shown to cause problems in reproductive behavior and development. Prior research has also linked abnormal development of the GnRH system in zebrafish to problems in reproduction and reproductive behavior. However, little research has been done on GnRH neuron development under stress in zebrafish. We primarily focused on the extrahypothalamic GnRH1 neurons located in the terminal nerve (TN), with a putative role in neuroendocrine and social regulation of reproduction. This study investigates effects of early, chronic cortisol exposure on the TN-GnRH1 neurons of embryonic zebrafish at 2 and 3 days post fertilization (dpf). Transgenic zebrafish embryos with GnRH1 neurons tagged with green fluorescent protein (GFP) were used to easily visualize embryonic changes to the developing reproductive neuroendocrine system. Embryos were exposed to a chronic dose of the stress hormone cortisol (hydrocortisone 10µM) by way of waterborne exposure in their embryo medium from 2 hours post fertilization (hpf) through 72 hpf. Embryos were fixed at 48 and 72 hpf, mounted in agar and their TN-GnRH1 neurons were examined using an upright epifluorescent microscope. The perimeters of individual neurons were marked in ImageJ and neuron area (in µm\textsuperscript{2}) and the short-diameter of the fitted ellipse (in µm) were calculated blind. Blind analysis of the images showed that embryos exposed to cortisol through 48 hpf (n=37 from 6 fish) showed a significant 23.50% reduction in the area of the individual neurons and a significant 13.54% reduction in the radius of the TN-GnRH1 neurons as compared to the control embryos (n=22 from 5 fish). Embryos exposed to cortisol through 3 dpf (n=36 from 7 fish) also showed a significant reduction, but to a lesser degree, of 10.80% in the area and 8.58% in the radius of individual GnRH1 neurons as compared to the control embryos (n=27 from 5 fish). Our study suggests that chronic exposure to stress in the early embryo delays or disrupts the
development of TN-GnRH1 neurons in zebrafish (Supported by NSF CAREER Award 1253126 to SR and Univ Puget Sound UEC Grant SR1563 to DS).

Disclosures: D. Skinner: None. S. Ramakrishnan: None.

Poster

523. Stress: Factors Affecting Sensitivity, Protection, and Recovery

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 523.30/W17

Topic: F.03. Motivation and Emotion

Support: US Army (W81XWH-12-0454)

Title: Effects of voluntary nicotine self-administration on fear conditioning in rats

Authors: E. RIDENER1, C. WEBBER1, C. W. ADAM2, K. STOLL2, E. MELONI1, S. B. CAINE2, *W. A. CARLEZON, Jr1;
1Dept Psychiat, Harvard Med. Sch./Mclean Hosp., Belmont, MA; 2ADARC, McLean Hosp., Belmont, MA

Abstract: Nicotine can facilitate learning and cognitive performance while also relieving feelings of stress. These two actions may have opposing effects on vulnerability to stress-related illness such as post-traumatic stress disorder (PTSD). The present experiments examined the effect of nicotine self-administration (SA) on the development and expression of PTSD-like symptoms in rats using the fear-potentiated startle (FPS) paradigm. FPS has elements that model an index trauma and enables quantification of exaggerated startle response and extinction deficits, two characteristics observed in humans with PTSD. Long-Evans rats were allowed to self-administer nicotine (0.03 mg/inj) or saline in 12hr (overnight) extended access sessions in standard operant conditioning chambers for a minimum of 14 sessions. Criteria for nicotine dependence was determined by SA of >0.7 mg/session for 4 out of 5 sessions and observable signs of spontaneous withdrawal 11.5 hrs post SA session. After criteria were met, rats were fear conditioned at one of two time points: either immediately after or 11.5 hrs after their last SA session. Fear conditioning consisted of 10 pairings of a 4-sec light (conditioned stimulus; CS) co-terminating with a 0.5-sec 0.6 mA footshock. Two different patterns of post-training nicotine intake were examined: for some rats, nicotine exposure was discontinued between fear conditioning and testing, whereas for others nicotine SA continued. At 10-12 days after fear conditioning, rats were tested immediately after SA three times, each test 48 hrs apart. Two metrics were examined in each of the test sessions: Context-potentiated startle (CPS) and Fear-
potentiated startle (FPS). %CPS was expressed as the percent change in startle after exposure to the conditioning context relative to a pre-training baseline. %FPS was expressed as the percent change in startle elicited in the presence of the light CS relative to trials without the CS. Rats that received fear conditioning immediately after SA sessions plus no further nicotine exposure showed reduced %CPS and normal %FPS, whereas those that continued nicotine SA showed normal %CPS but reduced %FPS. In contrast, rats that were fear conditioned during nicotine withdrawal plus no further nicotine showed elevated %CPS and normal %FPS. Rats that received fear conditioning during nicotine withdrawal plus continued nicotine SA also showed enhanced CPS, but reduced %FPS as well as enhanced extinction. Our data suggest that, under certain conditions, nicotine can reduce behavioral responsiveness to cues associated with a stressful (trauma-like) event, whereas nicotine withdrawal can enhance these same metrics.

between dopamine regulation and motivational behaviors and their implications for sleep regulation.

**Disclosures:** A. Pavin: None. D. Sitaraman: None.

**Poster**

**524. Food Intake and Energy Balance: Monoamines and Other Regulators**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 524.02/W19

**Topic:** E.07. Food Intake and Energy Balance

**Title:** Central mechanism of rosiglitazone induced food intake

**Authors:** *J. MATIAS, E. R. GILBERT, M. A. CLINE; Virginia Tech., Blacksburg, VA

**Abstract:** Rosiglitazone, a thiazolidinedione, is a peroxisome proliferator-activated receptor gamma (PPARγ) synthetic activator that increases insulin sensitivity. A documented side effect of this diabetes drug is increased appetite, although the mechanism mediating this response is unknown. To better understand effects on food intake regulation, we evaluated the appetite-associated effects of rosiglitazone in an alternative vertebrate and agriculturally-relevant model, the domesticated chick. Four day-old chicks received intracerebroventricular (ICV) injections of 0, 5, 10 or 20 nmol rosiglitazone and food and water intake were measured. Only chicks that received 20 nmol rosiglitazone increased food intake during the 2 hour observation period, with no effect on water intake. In the next experiment, chicks were ICV-injected with 20 nmol rosiglitazone and hypothalamus was collected at 1 hour post-injection for RNA isolation. Real-time PCR was performed to measure mRNA abundance of appetite associated factors. Neuropeptide Y (NPY) and proopiomelanocortin (POMC) mRNA decreased while NPY receptor 1 mRNA increased in rosiglitazone-injected chicks compared to the controls. Results show that central effects of rosiglitazone on appetite are conserved between birds and mammals, and that increases in food intake might be mediated through NPY and POMC neurons in the hypothalamus.

**Disclosures:** J. Matias: None. E.R. Gilbert: None. M.A. Cline: None.
Title: The interaction of binge-eating and stress-responsivity in mice

Authors: *T. A. Czyzyk*{superscript}1,2, T. M. Tang{superscript}2, N. K. Barker{superscript}2, A. N. Polito{superscript}2, P. A. Smith{superscript}2, J. Krantz{superscript}2, Y. E. Geda{superscript}2, P. Telenisky{superscript}2;{superscript}1Anesthesiol., Penn State Univ. Col. of Med., Hershey, PA;{superscript}2Metabolic Hlth. Program, Mayo Clin. Arizona, Scottsdale, AZ

Abstract: Prolonged stress in childhood is associated with binge-eating disorder (BED) in humans, and it is thought that stress can precipitate binge-like eating episodes. We used a novel mouse model of binge-like eating behavior to better understand how glucocorticoids may be involved in the initiation and maintenance of binge-eating. C57BL/6 mice were randomized to three groups: low-fat chow diet only (chow group), unrestricted access to both energy dense, high fat diet (HED, 40% fat) and chow (HED group), or unrestricted access to chow plus access to HED during one 24h period/week (binge group) (Czyzyk, et al. 2010, Obesity, 18: 1710). Serum corticosterone levels (CORT) were measured before, during and after the initiation of binge-eating. CORT was similar across all groups 2h prior to, and 1h after the binge group had access to HED. Compared to controls, CORT was significantly elevated (double) in the binge group 6h after access to HED, but returned to baseline by 20h post-binge. Next, male and female mice with a 52 wk history of bingeing were subjected to 5 min of acute restraint stress prior to sacrifice and measurement of CORT. Basal CORT was significantly elevated in female, but not male mice of the binge group. Acute restraint stress increased CORT 4-8 times in mice of the chow and HED groups; however, neither males nor females of the binge group had elevated CORT in response to this stress. This blunted CORT response to acute stress was not observed after only 14 wk of binging. In a behavioral model of stress-responsivity, male mice in the binge and HED groups had significantly increased locomotor activity after a 2h restraint period compared to the chow group. However, female mice did not show this diet-dependent stress-induced hyperlocomotion after 6 wk of binge-eating. Lastly, mice were exposed to maternal separation stress followed by regular exposure to non-littermates (adolescent stress), and then 12 wk of binge-eating. Early-life stress did not overtly change the pattern of HED intake. However,
the combination of early-life stress and a history of bingeing significantly increased basal CORT in females, but not in males. Collectively, our data suggest that 1) elevated CORT after bingeing might drive subsequent overeating by decreasing the compensatory reductions in night-time food intake which has been observed in other rodent models of binge-eating, 2) the hormonal response to an acute stressor is blunted with prolonged binge eating, 3) there is an interaction between binge-eating and early life stress exposure, and 4) there are sex-specific differences in these responses which are consistent with the difference in prevalence of BED between human males and females.


Poster

524. Food Intake and Energy Balance: Monoamines and Other Regulators

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 524.04/W21

Topic: E.07. Food Intake and Energy Balance

Support: CONCYTEG Grant 07-16-k662-054

CONACYT Grant 162016

Title: Effect of strawberry extracts on GABA concentration and oxidative stress in the frontal cortex of rats high fat diet fed

Authors: *C. SANDOVAL SALAZAR*¹, C. OVIEDO-SOLÍS², S. SOLÍS-ORTÍZ³, H. AGUILAR-ZAVALA⁴, V. BELTRAN-CAMPOS⁴, J. RAMIREZ-EMILIANO⁵; ¹Univ. De Guanajuato, Celaya, Mexico; ²Medicina y Nutricion, ³Ciencias Médicas, Univ. De Guanajuato, Leon, Mexico; ⁴Ciencias de la Salud e Ingenierias, Univ. De Guanajuato, Celaya, Mexico

Abstract: Obesity is associated with psychological factors, genetic predisposition and food habits. The inhibitor neurotransmitter γ-aminobutíric acid (GABA) and the frontal cortex have a role in food intake. Some studies reported that chronic intake of a high-fat diet produce changes in GABA concentration and oxidative stress which accompanied by accelerate lipid peroxidation. Natural products, such as flavonoids found in fruits like strawberry have potential therapeutic agents recognized for their antioxidant activity. The aim of this study was to explore the effect of antioxidant strawberry no irradiated (NR) and irradiated (R) extract on the GABA
concentration, lipids damage oxidative in the frontal cortex of rats high-fat diet fed. A total of 40 healthy male rats were divided equally into four groups, the first group was fed with standard diet (SD) and the others groups was fed with high-fat diet (HF) during three months. Subsequently HF groups were separated in three groups (HF, HF-NR and HF-R) which were fed for eight weeks and received strawberry extracts. Food intake was recorded daily. Biochemical parameters was acquired using enzymatic methods. The GABA level was quantified using high-performance liquid chromatography (HPLC). Lipid peroxidation levels were analyzed by measuring thiobarbituric acid reactive substances. The SD-fed rat intake was approximately 30.4% more than the HF, HF-NR and HF-R. The glucose levels of the SD-fed rats were significantly different from the HFD-groups. The strawberry extracts NR and R decreased the cholesterol levels as the SD group. The lipid peroxidation in the frontal cortex were high in HF and HF-NR with significant difference compared with SD group. The strawberry extract R treatment decreased TBARS levels in frontal cortex. The GABA levels in the HF-fed rats demonstrated a significant decrease compared with the SD group in the frontal cortex. The GABA levels in the HF-NR group treated with strawberry NSE extract appeared to be more elevated without reaching to SD group. This results indicate that exposure to hypercaloric diet decrease the GABA concentration and increase oxidative stress in the frontal cortex, suggesting a disturbance in inhibitory process of food intake.

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**Poster**

**524. Food Intake and Energy Balance: Monoamines and Other Regulators**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#: 524.05/W22

**Topic:** E.07. Food Intake and Energy Balance

**Support:** PSC/CUNY Grant 41-63428

- PSC/CUNY Grant 42-336
- PSC/CUNY Grant 45-67301

**Title:** NMDA antagonism and acquisition and expression of fat-conditioned flavor preferences in BALB/c and SWR mice
Authors: *T. T. KRAFT*¹,², D. HUANG², S. LAMAGNA², D. WARSHAW², E. NATANOVA², A. SCLAFLANI³, R. J. BODNAR²;
¹Home, Englewood, NJ; ²Psychology, Queens College, CUNY, Flushing, NY; ³Psychology, Brooklyn College, CUNY, Brooklyn, NY

Abstract: Many forms of appetitive learning are modulated by glutamatergic systems. Both sugar (e.g., sucrose, fructose) and fats (e.g., corn oil, Intralipid) can condition flavor preferences (CFP) in rats and mice with previously-observed N-methyl-D-aspartate (NMDA) receptor mediation. The acquisition, but not expression, of fructose-CFP is eliminated by systemic NMDA receptor antagonism (MK-801) in rats. BALB/c mice are more sensitive to MK-801 than SWR mice in eliminating acquisition of both sucrose- and fructose-CFP. Expression of fructose-, but not sucrose-CFP was significantly, but marginally reduced by MK-801 in SWR, but not BALB/c mice. Fat-CFP can also be elicited by pairing distinctive flavors (e.g., grape, cherry Kool-Aid) with two concentrations of corn oil in rats and emulsified Intralipid in mice in training and testing for preferences. Like sugar-CFP, acquisition of fat-CFP is eliminated by NMDA receptor antagonism in rats, whereas expression is marginally affected at high doses. The present study examined whether NMDA receptor antagonism of the acquisition and expression of fat-CFP is subject to genetic variance in BALB/c and SWR mice. Food-restricted BALB/c and SWR mice drank flavored 5% (CS+, e.g., cherry Kool Aid) and 0.5% (CS−, e.g., grape Kool Aid) Intralipid solutions in ten alternating (1 h/day) training sessions followed by two-bottle choice tests with both CS+ and CS− flavors presented in 0.5% Intralipid. In expression, vehicle (Veh) and MK-801 doses of 100 and 200 ug/kg were administered prior to two-bottle testing. Expression of fat-CFP was significantly reduced by both MK-801 doses in BALB/c (48-50%) and SWR (48-62%) mice relative to Veh preferences (71-72%), indicating the elimination of fat-CFP in both strains. In acquisition, separate groups of BALB/c and SWR mice received Veh or MK-801 (100 ug/kg) injections prior to the daily one-bottle CS+ and CS− training sessions. Six two-bottle CS+ vs. CS− choice tests were then conducted. Fat-CFP acquisition was eliminated by MK-801 in both BALB/c and SWR mice across the three pairs of tests. These data demonstrate that both murine strains require the full integrity of NMDA receptors for the acquisition (learning) and expression (maintenance) of flavor preferences elicited by fat.


Poster

524. Food Intake and Energy Balance: Monoamines and Other Regulators

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM
**Program#/Poster#:** 524.06/W23

**Topic:** E.07. Food Intake and Energy Balance

**Title:** The role of lateral septum opioid receptors in feeding behavior

**Authors:** *M. CALDERWOOD, B. STANLEY;
Dept. of Psychology, Univ. of California Riverside, Riverside, CA

**Abstract:** Opioid peptides and receptor systems are known to be involved in the modulation and control of feeding behavior (Bodnar, 2004). Morphine, a μ opioid receptor agonist, elicits eating in rats when injected into the multiple brain regions related to feeding and reward including the nucleus accumbens shell (AcbSh) and multiple regions of the hypothalamus (Castro & Berridge, 2014; Stanley et al. 1988). Interestingly morphine produces a particularly large feeding effect when injected into the lateral septum (LS). The mechanisms underlying this septal effect are unknown and the purpose of this study was to address that deficiency. Our replication of Stanley et al., (1988) and found that feeding is elicited by morphine in lateral septum at a range of doses including 5, 8, 17 and 34 μg/.3μl. We investigated the site specificity of this effect by injecting morphine into multiple brain sites bracketing the LS and found significant and robust effects in the LS and ventral LS. Subsequently, we used the immediate early gene cFos in order to identify the pathways involved. We hypothesized that there would be significantly more cFos seen in the AcbSh and ventral tegmental area (VTA). We also hypothesized that there would be increased cFos in the lateral hypothalamus, a brain region known to be involved in feeding and which has direct connections to and from the LS (Risold & Swanson, 1997). We found no significant differences in cFos between morphine and control animals in the AbSh or the VTA. However we did find significantly increased cFos activation in the lateral hypothalamus as well as the septohypothalamic nucleus at 120 and 180 minutes post injection but not 90 minutes post injection for morphine animals compared to control animals. This suggests an important role for the lateral hypothalamus in this feeding effect and potentially a more complex role for opioids in the lateral septum in modulating feeding.

**Disclosures:** M. Calderwood: None. B. Stanley: None.

**Poster**

524. **Food Intake and Energy Balance: Monoamines and Other Regulators**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 524.07/W24

**Topic:** E.07. Food Intake and Energy Balance
**Support:** New Brunswick Foundation for Health Research

Mount Allison University Internal Grant

**Title:** Endocannabinoids and nitric oxide interactions in stress-induced feeding

**Authors:** *K. M. CROSBY*¹, J. THEBEAU¹, N. COCHKANOFF¹, A. SMITHERS², T. BROOKS¹;

¹Biol., ²Chem. and Biochem., Mount Allison Univ., Sackville, NB, Canada

**Abstract:** Stressful stimuli trigger rapid and requisite changes that allow organisms to cope with stress and restore homeostasis. While this response can be beneficial in the short term, prolonged exposure to stress can result in long-lasting pathophysiological consequences including obesity. The mechanisms underlying the link between stress and obesity; however, are poorly understood. Numerous feeding circuits in the brain play an important role in regulating appetite and body weight, but no region is as fundamentally linked to stress and appetite as the dorsomedial nucleus of the hypothalamus (DMH). Within the DMH, communication between putative satiety neurons is modulated by two signaling molecules: endocannabinoids (eCBs) and nitric oxide (NO). Recent electrophysiological studies have revealed that eCBs and NO interact to influence synaptic activity in the DMH. It remains unknown, however, whether these *in vitro* interactions translate into *in vivo* effects on food intake and how stress alters these signals to promote obesity. Thus, the purpose of this study was to examine if eCBs and NO interact to influence food intake, and whether this interaction is disrupted by prolonged exposure to stress. Male Sprague Dawley rats received various combinations of drugs to activate or inhibit eCB and NO signaling either into the periphery or directly into the DMH. Following drug administration, food intake and body weight were measured and brains collected to assess neuronal activity through immunohistochemistry studies. To examine the effect of stress, separate groups of animals were subjected to restraint stress, and the experiments repeated as outlined above following the final episode of stress. Overall, we show that eCBs and NO interact in the DMH to modulate food intake, and this effect is influenced by stress. These findings may provide important insight into the mechanisms that underlie stress-induced feeding.

**Disclosures:** K.M. Crosby: None. J. Thebeau: None. N. Cochkanoff: None. A. Smithers: None. T. Brooks: None.

**Poster**

524. Food Intake and Energy Balance: Monoamines and Other Regulators

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM
Title: The endocannabinoid system into the Prelimbic Cortex modulates food intake in rats
Authors: *A. A. SCOPINHO¹, L. B. M. RESSTEL², F. M. A. CORRÊA²;
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Abstract: Introduction: The endocannabinoid system is involved in the central regulation of feeding behavior. Systemic administration of exogenous cannabinoids or endocannabinoids stimulates eating, and systemic administration of CB1 antagonist attenuates agonists’ stimulatory effects on food intake, suggesting the involvement of these receptors in food intake modulation. Although CB1 receptors are present principally in the central nervous system (CNS), little is known about which areas are involved in this modulation and the role of endocannabinoids system. Therefore, the aim of the present study is to investigate the effects evoked by CB1 receptors antagonist (AM251) and fatty acid amide hydrolase (FAAH) inhibitor URB597 microinjected in the prelimbic cortex (PL) on the food intake regulation. Methods: CB1 antagonist AM251 (10, 50, 100pmol/100nl), FAAH inhibitor (URB597) or aCSF- artificial cerebrospinal fluid were microinjected into the PL of fed or fasted Wistar rats and 10 min later, the food intake test was performed during one hour for food intake determination. Results: The amount of food ingested by fasted animals was significantly higher than fed animals ($F_{(1, 48)} = 482.2, P<0.001$). Significant effects of treatment with doses of AM251 ($F_{(3, 46)} = 74.41, P<0.001$) and interaction between the two factors ($F_{(3, 48)} = 78.26, P<0.001$) for total food intake (n=6-8 each group) were observed only in fasted rats. Moreover, significant effects of treatment with doses of URB597 ($F_{(3, 46)} = 23.56, P<0.001$) and interaction between the two factors ($F_{(3, 46)} = 7.075, P=0.0006$) for total food intake (n=6-8 each group) were observed only in fed rats. Conclusion: The blockade of PL CB1 receptors inhibited food intake in fasted animals, and inhibition of FAAH into the PL increased the amount of food ingested in fed rats, suggesting that PL endocannabinoid system modulated food intake behavior and satiation with involvement of anandamide and CB1 receptors.
Disclosures: A.A. Scopinho: None. L.B.M. Resstel: None. F.M.A. Corrêa: None.
Poster
Abstract: Obesity is defined as excess body fat and it represents a public health problem in adults and children around the world. This disease is the result of several factors, including consumption of food that has little nutritional value known as “junk food”. Current evidence has shown that feeding animals with junky food induces behavior, neurochemical and molecular changes. In this regard, we have reported previously that rats under cafeteria diet protocol displayed chocolate preference as well as an enhancement in mitogen-activated protein kinase expression in hypothalamus. To test whether intrahypothalamic microinjection of 8-hydroxy-2-(di-n-propylamino)tetratin (8-OH-DPAT), a serotonin (5-HT1A) receptor agonist, would block chocolate feeding, rats were fed with respective item during 30 days. Next, animals were implanted with a cannulae aimed to the lateral hypothalamus as well as a microdialysis guide-cannula placed into the nucleus accumbens core. After 7 days of recovery from surgical procedures, pharmacological challenges were carried out. Microdialysis samples were analyzed to determine the levels of serotonin (5-HT) by HPLC means. All rats received either a microinjection of saline (control group) or 8-OH-DPAT (10µg/1µL) into the lateral hypothalamus and microdialysis samples were collected hourly. We found that administration of 8-OH-DPAT increased chocolate-intake. Moreover, injection of 8-OH-DPAT in rats fed with chocolate did not modify the contents of 5-HT. The current results suggest that consumption of chocolate involve serotonergic receptors presumably placed in hypothalamus. However, it seems that 5-HT is not involved in chocolate-intake. Further studies are needed to elucidate the molecular mechanisms of craving junky food.

Poster

524. Food Intake and Energy Balance: Monoamines and Other Regulators

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 524.10/W27

Topic: E.07. Food Intake and Energy Balance

Support: NHMRC Grant App1063955

NHMRC Grant App1079233

Title: Long chain fatty acids regulate electrical activity of NPY and POMC neurons in the arcuate nucleus

Authors: N. J. MICHAEL\textsuperscript{1}, M. VAN DEN TOP\textsuperscript{2}, F.-Y. ZHAO\textsuperscript{2}, V. R. HAYNES\textsuperscript{1}, M. WATT\textsuperscript{1}, *D. SPANSWICK\textsuperscript{2};
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Abstract: The brain primarily utilises glucose as a source of energy, however, free fatty acids and ketones can serve as an alternate energy source under certain conditions. In addition to acting as an alternate source of energy for the brain, fatty acids have been shown to act centrally to modulate food intake, body weight, and glucose production. Previous studies suggest fatty acids, including long chain fatty acids, act as signalling molecules to some hypothalamic neurons, which may explain the changes in feeding and bodyweight observed with fatty acid administration. We sought to investigate whether the long chain fatty acids, oleic acid and palmitic acid, could regulate the electrical activity of Neuropeptide Y (NPY) and Proopiomelanocortin (POMC) neurons within the arcuate nucleus of the hypothalamus; key neuronal populations known to control energy homeostasis. Whole-cell patch clamp electrophysiology was performed on hypothalamic slices that contained the arcuate nucleus from mice expressing green fluorescent protein in NPY or POMC neurons. Bath application of oleic acid (5\textmu M) was found to excite 36\% (9/25) and inhibit 20\% (5/25) of NPY neurons. In POMC neurons, oleic acid primarily induced an inhibitory response (50\%, 18/36), however, 11\% (4/36) of these cells were excited. Palmitic acid (40\textmu M) also displayed both excitatory (22\%, 5/23) and inhibitory (26\%, 6/23) effects on NPY neurons. The effects of palmitic acid on POMC neurons mirrored those of oleic acid. The majority of the POMC neurons were inhibited by palmitic acid.
(43%, 9/21), and a small proportion of these cells were excited (9%, 2/21). These results suggest that long chain fatty acids may exert their effects on energy homeostasis through direct regulation of NPY and POMC neuronal activity.


Poster

524. Food Intake and Energy Balance: Monoamines and Other Regulators

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 524.11/W28

Topic: E.07. Food Intake and Energy Balance

Support: PSC/CUNY Grant 41-3428,

PSC/CUNY Grant 42-336

PSC/CUNY Grant 43-232

PSC/CUNY Grant 45-67301

Title: Muscarinic and nicotinic cholinergic receptor antagonists differentially mediate rat acquisition of fructose-conditioned flavor preference and quinine-conditioned flavor avoidance

Authors: *F. M. ROTELLA, K. OLSSON, V. VIG, J. PAGIRSKY, I. YENKO, I. KOHEN, A. AMINOV, T. DINDYAL, R. J. BODNAR; Psychology, Queens College, CUNY, Flushing, NY

Abstract: Rats display both conditioned flavor preference (CFP) to fructose, and conditioned flavor avoidance (CFA) following sweet adulteration with quinine. Previous studies indicate that whereas acquisition of fructose-CFP was significantly reduced by dopamine (DA) D1, DA D2 or NMDA, but not opioid antagonists, acquisition of quinine-CFA was significantly enhanced and prolonged by DA D1, NMDA or opioid, but not DA D2 antagonists. Both muscarinic and nicotinic cholinergic receptor signaling have been implicated in sweet intake and development of food-related preferences. Moreover, muscarinic (scopolamine: SCOP) or nicotinic (mecamylamine: MEC) cholinergic receptor antagonists decreased expression of fructose-CFP. The present study examined the effects of SCOP and MEC on fructose-CFP acquisition and quinine-CFA acquisition. In fructose-CFP, six groups of rats received vehicle, SCOP (1 or 2.5 mg/kg), MEC (4 or 6 mg/kg) or a limited intake control 0.5 h prior to 10 CS+ (8% fructose and
0.2% saccharin) and CS- (0.2% saccharin) training sessions followed by six 2-bottle CS+ and CS- choice tests in 0.2% saccharin. For quinine-CFA acquisition, five groups of rats received vehicle, SCOP (1 or 2.5 mg/kg) or MEC (4 or 6 mg/kg) 0.5 h prior to 8 one-bottle CS- (8% fructose + 0.2% saccharin: FS) and CS+ (fructose + saccharin + quinine (0.030%: FSQ) training sessions followed by six 2-bottle CS- and CS+ choice tests in fructose-saccharin solutions. Fructose-CFP acquisition was eliminated by SCOP at doses of 1 (40-54%) and 2.5 (45-58%) mg/kg accompanied by a failure to observe CS+ and CS- intake differences during testing relative to vehicle (85-92%) and limited control (74-88%) conditions. In contrast, MEC failed to alter fructose-CFP acquisition. Quinine-CFA acquisition was significantly enhanced and prolonged by MEC at 4 (18-24%) and 6 (11-13%) mg/kg relative to vehicle (34-48%). In contrast, SCOP failed to alter quinine-CFA acquisition. These data implicate the cholinergic receptor system in mediating acquisition (learning) of sugar-induced preferences and quinine-induced aversions with muscarinic receptor signaling controlling the former and nicotinic receptor signaling controlling the latter.


Poster

524. Food Intake and Energy Balance: Monoamines and Other Regulators

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 524.12/W29

Topic: E.07. Food Intake and Energy Balance

Support: NSERC

Title: Rapamycin increases cognition and protects against the depressive symptoms associated with chronic food restriction

Authors: *T. KENNY, M. HEBERT, P. MACCALLUM, J. WHITEMAN, G. MARTIN, J. BLUNDELL; Mem. Univ. of Newfoundland, St. John's, NL, Canada

Abstract: Anorexia nervosa (AN) is characterized by insufficient food intake leading to persistently lowered body weight and is frequently comorbid with anxiety, depression and cognitive deficits. Currently, research into the etiology of anorexia nervosa (AN) is limited by the inability of animal models to produce both its core symptoms and comorbidities. Recent data suggests that a single systemic injection of the mTOR inhibitor rapamycin (RAP) decreases food
intake for several days and lowers body weight for at least 10 weeks, mimicking the core symptoms of AN. Furthermore, preliminary data from our lab suggests that the meal patterning induced by RAP resembles that seen in AN. Thus, the goal of our study was to examine whether RAP could also produce the comorbidities of AN yielding a more comprehensive animal model of AN. We assessed anxious, depressive and cognitive behaviours during the period of decreased food intake following a single injection of RAP (1-7 days post injection), as well as 6 weeks later when food intake was normalized yet body weight remained lowered relative to vehicle-injected control animals. Anxiety-like behaviour was measured in the elevated plus maze, depressive-like behaviours were measured in the forced swim test, and cognition was measured in the novel object recognition task. To ensure that any differences in behaviour were not the result of decreased food intake or body weight alone, we included a vehicle-injected yoked control group who had their food intake matched to that consumed by their RAP-treated counterparts.

Consistent with past research, a single injection of RAP decreased food intake for six days and lowered body weight for at least 57 days in rats. Interestingly, RAP improved cognition and reduced caloric restriction-induced depressive-like behaviors in the first week following injection, without altering anxiety-like behaviour or locomotor activity. Our data suggest that RAP does not produce an animal model of AN, rather it may protect against the cognitive and depressive symptoms associated with chronic food restriction. Follow-up studies are underway to examine the influence of RAP on calorie restriction-induced depressive-like behaviours and cognitive impairments. Given the pattern of decreased food intake, increased cognition and decreased depression we propose that RAP acts by upregulating serotonin via the inhibition of interleukin-2. Although our hypothesis that a single injection of RAP may act as an animal model of AN was not supported, the findings suggest that RAP may instead represent a viable option in obesity treatment.

Title: Nitric oxide produces excitation and inhibition of feeding in satiated rats via different mechanisms

Authors: N. HAZUT1, A. WELLER2, *A. J. SUSSWEIN3;
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Abstract: Previous data by others has shown that nitric oxide (NO) mediates some of the orexic effects of ghrelin and PYY, and therefore it is “known” that NO excites feeding. However, we have recently found that NO is an inhibitor of feeding when rats are satiated, but nonetheless occasionally snack on chow. In this condition, treatment with a competitive inhibitor of L-arginine, the precursor from which NO is synthesized, caused increased snacking, and treatment with an NO donor caused decreased snacking. Treatment with L-arginine also inhibited snacking on chow. However, when satiated animals were offered a high-fat attractive food, rather than chow, the competitive inhibitor caused inhibition of snacking, rather than exciting it. Thus, NO has both excitatory and inhibitory effects in satiated animals, based on the palatability of the snack. NO inhibiting snacking is likely released as a result of satiating signals, whereas NO exciting snacking is released in response to attractive food. A fine-grained examination of the individual bouts of snacking showed that excitatory and inhibitory effects of NO are qualitatively different. NO inhibition regulates the number of bouts, with the NO donor and L-arginine decreasing the number of bouts, and the NO inhibitor increasing them. There were no effects on bout length or bout efficacy. By contrast, with high-fat food the NO inhibitor reduced the efficacy of bouts, without affecting their length or frequency. These data suggest that NO excitation and inhibition of feeding are via different mechanisms, probably localized to different neural sites. Excitation is via effects on bout efficacy, whereas inhibition is via bout number. The effects of an NO donor and of L-arginine in satiated animals challenged with a high fat food were not mirror images of the effect of the NO inhibitor. The NO blocker and exogenous NO and L-arginine all had primarily inhibitory effects on snacking on the high fat diet, the former by reducing bout efficacy, and the latter by reducing number of bouts. These results emphasize the dual effects of NO on snacking.


Poster

524. Food Intake and Energy Balance: Monoamines and Other Regulators

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 524.14/W31

Topic: E.07. Food Intake and Energy Balance
Title: Influence of glycine in food and water intake, body weight, transaminases levels and sleep architecture in rats

Authors: F. ALBERTO-PATRICIO¹, S. A. ZAVALA-RIVAS¹, A. K. LEON-OLGUIN¹, I. JASSO-VILLAGOMEZ², G. BLANCAS-FLORES², J. VELAZQUEZ-MOCTEZUMA¹, *A. JIMENEZ-ANGUIANO³; ¹Area de Neurociencias, Depto. Biologia de la Reproduccion, ²Area de Investigacion Medica, Depto. de Ciencias de la Salud, ³Univ. Autonoma Metropolitana-Iztapalapa, MEXICO City, Mexico

Abstract: Glycine (Gly) is an amino acid involved as an anti-inflammatory that protects against disease states in animal models of ischemia-reperfusion and injury; attenuates the increase in free fatty acids and in treatment and prevention of type 2 diabetes mellitus. Gly also acts as an inhibitory neurotransmitter of the motor neurons and mediates the post-synaptic inhibition responsible for REM sleep atonia. The administration of Gly improves the quality of sleep and reduces daytime sleepiness. However, the temporary effect of the administration of Gly on metabolism and sleep is unknown. We used 16 male rats (200-250 g), under the following conditions: Control, normal water consumed (n=8) and water with Gly (10 g/1 L) for 60 days (n=8). In the first part of this study, we evaluated the effect of chronic administration of Gly quantified every third day: body weight, food and water intake of the animals. At the beginning and at the end of treatment of Gly, we measured in samples of blood: Transaminases leves, triglycerides and glucose. In the second part, we evaluated the effect of chronic administration of Gly on sleep pattern. Electrodes were implanted for conventional sleep recordings. Once a week for one month, we realized polysomnographic recordings. Results showed that chronic administration of Gly decrease transaminases levels, food intake and body weight in relation with the control group. In sleep, Gly promoted in the first three weeks an increase in slow-wave-sleep II (SWS II) decreasing REM sleep, in relation to the control group. In the fourth week, Gly caused a gradual decrease in SWS II, with an increase in SWS I. From the gotten results we can suggest a benefical effect of Gly in the metabolism and a dual effect on sleep dependent of the time administration.


Poster

524. Food Intake and Energy Balance: Monoamines and Other Regulators

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM
Energy balance regulation by GABA neurons of the lateral hypothalamus

Authors: *C. Kosse, P. Iordanidou, D. Burdakov;
The Francis Crick Inst., London, United Kingdom

Abstract: The brain’s ability to control body weight is under intense investigation, and requires the lateral hypothalamus (LH). This ability of the LH has been historically attributed to weight-gain promoting LH neurons containing melanin-concentrating hormone and weight-loss-promoting neurons containing orexin/hypocretin. However, recent investigations revealed a novel subpopulation of LH neurons that are orexin- and MCH-negative, but contain GABA. We have investigated the function of these GABAergic LH neurons using pharmacogenetic tools. We found that modulating LH GABAergic neurons \textit{in vivo} regulates body weight to the same extent as previously reported for MCH neurons. Surprisingly, food intake was not affected by activation of LH GABAergic neurons, suggesting a mechanism of weight control independent of diet. Furthermore, we used optogenetic-assisted circuit mapping to probe connections between LH GABAergic neurons and body-weight-increasing NPY cells in the hypothalamic arcuate nucleus. However, we did not find any evidence for inhibitory control of arcuate NPY cells by LH GABA neurons. Our data elucidate the function of a new controller of diet-independent hypothalamic control of energy balance, opening further avenues for dissecting neurophysiological mechanisms of weight loss.

Disclosures: C. Kosse: None. P. Iordanidou: None. D. Burdakov: None.
Title: Treating obesity and related metabolic disorders through a sympathetic approach

Authors: *C. Yang*, L. Sipe, J. Hirsh, C. Deppmann; Univ. of Virginia, Charlottesville, VA

Abstract: Obesity, along with its many associated health disorders, is one of the foremost healthcare issues facing the modern world. In attempts to stave the ongoing increases in obesity rates, a number of therapies for this affliction have been developed, many of which have produced limited results in reducing obesity rates. Here, we suggest a new avenue for treating obesity by targeting the sympathetic nervous system. We report that wild-type mice placed on weight loss-inducing ketogenic diets exhibit fluctuations in the release of norepinephrine by sympathetic neurons to both white and brown adipose tissue. These fluctuations in norepinephrine appear to correspond to periods of weight loss and stoppage of weight loss across a 12 day time period. Moreover, we show that application of β3-adrenergic receptor agonists enhance weight loss in response to the ketogenic diet while the respective antagonists or complete ablation of the sympathetic nervous system can reduce weight loss responses. These data provide a foundational framework for developing novel therapies to treat obesity.

Disclosures: *C. Yang*: None. *L. Sipe*: None. *J. Hirsh*: A. Employment/Salary (full or part-time); University of Virginia. *C. Deppmann*: A. Employment/Salary (full or part-time); University of Virginia.

Poster

524. Food Intake and Energy Balance: Monoamines and Other Regulators

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 524.17/W34

Topic: E.07. Food Intake and Energy Balance

Support: PAPIIT IN 224214

PAPCA -2014-54

PAEP

Title: Feeding effect of 5-HT1A receptor stimulation on PVN with and without inhibition of HPA axis
Authors: *M. RITO, V. LÓPEZ ALONSO, K. REYES SANTOS, G. AMBROSIO SEGUNDO, K. CRUZ GARCÍA, J. MANCILLA DÍAZ; Univ. Nacional Autonoma De México, México, Mexico

Abstract: Paraventricular nucleus of the hypothalamus (PVN) expressed 5-HT1A receptor in parvocellular area where is located neurons that synthetize and release corticotropin release hormone (CRH). It has been described that stimulation of 5-HT1A receptor on PVN produce an increase of CRH, adrenocorticotropic hormone and corticosterone in rats due stimulation of hypothalamic pituitary adrenal axis (HPA). The aim of the present study was characterize the effect of 5-HT1A receptor stimulation on PVN with or without inhibition of HPA axis. Male Wistar rats of 250-300 g were accustomed to a diet of separate sources of protein, carbohydrate and fat, then a cannula were implanted on right PVN and a second surgery to remove the adrenal glands bilaterally (ADX) or sham surgery (sham). After the post-surgery recovery period of 5 days, the rats were assigned to a group and a treatment, sham (salt solution), sham+8-OH-DPAT, sham+WAY100635, sham+WAY100635+8-OH-DPAT, ADX (salt solution), ADX+8-OH-DPAT, ADX+WAY 100635, ADX+WAY 100635+8-OH-DPAT. 60 min record with continuous duration was made for behavioral satiety sequence (BSS) analysis. The results showed that adrenalectomy decrease food intake. Administration of 8-OH-DPAT decrease total food intake and carbohydrates in sham groups. Pretreatment with WAY 100635 prevent hypophagic effect induce by 8-OH-DPAT. BSS reveal that 8-OH-DPAT delay the natural development pattern of satiety at the beginning of dark phase. Administration 8-OH-DPAT increases Glucose serum concentration. Agonist 8-OH-DPAT decrease food intake of carbohydrates in ADX groups. Adrenalectomized rats interrupt the natural pattern of BSS. Finally is possible to conclude that HPA axis must be intact for integration of serotonergic and CRH signals to regulate food intake and BSS.

Disclosures: M. Rito: C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); PAEP. Other; Posgrado Ciencias Biológicas UNAM. V. López Alonso: None. K. Reyes Santos: None. G. Ambrosio Segundo: None. K. Cruz García: None. J. Mancilla Díaz: None.

Poster

524. Food Intake and Energy Balance: Monoamines and Other Regulators

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 524.18/W35

Topic: E.07. Food Intake and Energy Balance
**Support:** PSC/CUNY Grant 41-3428

PSC/CUNY Grant 42-336

PSC/CUNY Grant 43-232

PSC/CUNY Grant 45-67301

**Title:** Baclofen, a GABA-B receptor agonist differentially mediates rat acquisition of fructose-conditioned flavor preference and quinine-conditioned flavor avoidance

**Authors:** K. OLSSON¹, F. M. ROTELLA², V. VIG¹, I. YENKO¹, J. PAGIRSKY¹, A. AMINOV¹, I. KOHEN¹, S. EHRENBERG¹, *R. J. BODNAR²;

¹Psychology, Queens College, CUNY, Flushing, NY; ²Psychology- Neuropsychology, Queens Col. & Grad Ctr, CUNY, Flushing, NY

**Abstract:** Rats display both conditioned flavor preference (CFP) to fructose, and conditioned flavor avoidance (CFA) following sweet adulteration with quinine. Previous studies indicate that whereas acquisition of fructose-CFP was significantly reduced by dopamine (DA) D1, DA D2 or NMDA, but not opioid antagonists, acquisition of quinine-CFA was significantly enhanced and prolonged by DA D1, NMDA or opioid, but not DA D2 antagonists. Activation of GABA-B receptors has been implicated in both the stimulation and inhibition of food intake, depending on the paradigm, route of administration and site of action. Recently, the GABA-B receptor agonist, baclofen (BAC) decreased expression of fructose-CFP. The present study examined the effects of BAC on fructose-CFP acquisition and quinine-CFA acquisition. In fructose-CFP, three groups of rats received vehicle or BAC at doses of 3 or 5 mg/kg 0.5 h prior to 10 CS+ (8% fructose and 0.2% saccharin) and CS- (0.2% saccharin) training sessions followed by six 2-bottle CS+ and CS- choice tests in 0.2% saccharin. For quinine-CFA acquisition, three groups of rats received vehicle or BAC at doses of 3 or 5 mg/kg 0.5 h prior to 8 one-bottle CS- (8% fructose + 0.2% saccharin: FS) and CS+ (fructose + saccharin + quinine (0.030%: FSQ) training sessions followed by six 2-bottle CS- and CS+ choice tests in fructose-saccharin solutions. The magnitude of fructose-CFP acquisition was reduced across the three pairs of choice tests by BAC at doses of 5 (71-76%), but not 3 (89-93%) mg/kg relative to vehicle (85-92%). Quinine-CFA acquisition transiently occurred in vehicle-trained rats after the first (34%), but not the subsequent two tests (47-48%). Whereas rats trained with the 3 mg/kg BAC dose showed stable quinine-CFA across all three tests (33-34%), rats trained with the 5 mg/kg BAC dose displayed significantly enhanced and prolonged quinine-CFA (15-25%). These data implicate the GABA-B receptor system in reducing acquisition (learning) of sugar-induced preferences and in enhancing quinine-induced aversions.

**Disclosures:** K. Olsson: None. F.M. Rotella: None. V. Vig: None. I. Yenko: None. J. Pagirsky: None. A. Aminov: None. I. Kohen: None. S. Ehrenberg: None. R.J. Bodnar: None.
Title: Effects of chronic central insulin infusion on food intake, glucose metabolism, and adiposity of male and female Wistar rats

Authors: *A. C. KISS*, M. C. D. DE MACEDO, D. W. DA SILVA, G. C. DE SOUZA, M. O. KLEIN, L. F. FELICIO, B. C. WOODSIDE

Abstract: High levels of insulin act on the brain decreasing the drive to eat. Research in animals showed that intracerebroventricular (ICV) administration of insulin decreases food intake, body weight, and adiposity. However most of these studies were conducted on males and it is well established that food intake on females varies according to estrous cycle phase and their reproductive state. There is evidence that male and female brains respond differently to ICV acute injection of insulin, with males being more sensitive to insulin catabolic effects. In a previous short experiment in our lab, the effect of chronic insulin ICV infusion on female rats was studied and although the dose used is known to decrease food intake in males, in females there was no difference. Therefore, the present study aimed to investigate the effects of chronic central insulin infusion on food intake, glucose metabolism, and adiposity of male and female Wistar rats. Female and male Wistar rats were randomly assigned to the following experimental groups: female saline (FS, n=10), female insulin (FI, n=10), male saline (MS, n=10), and male insulin (MI, n=10). Female rats from FS and FI groups were ovariectomized on postnatal day (PND) 75, 15 days before cannula placement surgery. All rats underwent surgery for cannula placement in the lateral ventricle around PND 90. Osmotic pumps delivered either saline or 10mU of insulin per day at a rate of 1µl/hour for 7 consecutive days. Body weight and food intake were recorded daily. Rats were submitted to glucose tolerance test. Body fat percentage was calculated. Unlike males, females did not present reduced food intake, body weight, or adiposity in response to ICV chronic insulin infusion. Our results reinforce the importance of...
studying sex difference response to the same treatment and add up to the demand to balance the use of males and females in research.


Poster

524. Food Intake and Energy Balance: Monoamines and Other Regulators

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 524.20/W37

Topic: E.07. Food Intake and Energy Balance

Title: The diet-induced obesity modifies the sensitivity of receptors CB1

Authors: *F. CORTÉS SALAZAR¹, O. J. SUÁREZ-ORTÍZ², D. DÍAZ-URBINA², J. M. MANCILLA-DÍAZ², R. E. ESCARTÍN-PÉREZ²;
¹Neurobiología de la Alimentación, ²Univ. Nacional Autónoma de México, Facultad De Estudios Superiores Iztacala, Mexico

Abstract: The CB1 receptors (RCB1) of the nucleus accumbens shell act as neuromodulators in coding reinforcing stimuli of food, especially when this is high-calorie. High consumption of this food is the major cause of obesity. That is why the aim of the study, is to evaluate the activation of CB1 receptors in rats fed for two months with a standard diet after being exposed to a high-calorie diet for five months. To a group of male rats of the strain Sprague Dawley, exposed for five months to a high-calorie diet (45% of lipid content, n = 4) and then expose them for two months with a standard diet (10% of lipid content). After spontaneous standard food consumption at the beginning of the dark phase was evaluated for two hours. Evaluations were made in subjects who were implanted with a cannula guide in the nucleus accumbens shell, was four treatments in a Latin square, with two days off between treatment. As agonist RCB1 the ACEA (0.5µg/µl) was used, as the antagonist AM281 (0.5µg/µl) was used. The results show that subjects previously exposed to five months of the calorie diet after two months of standard diet are more sensitive than controls.


Poster
Intrahypothalamic injection of SCH 23390 enhances chocolate intake but does not modify extracellular levels of dopamine in nucleus accumbens in rats

Authors: *A. SARRO-RAMIREZ*, J. PASTRANA-TREJO†, G. ARANKOWSKY‡, E. MURILLLO-RODRIGUEZ1,2,3;  
†Grupo de Investigación en Envejecimiento, ‡Grupo de Investigación Desarrollos Tecnológicos para la Salud División de Ingeniería y Ciencias Exac, †Univ. Anahuac Mayab, Merida, Mexico; ‡Ctr. de Investigaciones Regionales Hideyo Noguchi, Univ. Autonoma de Yucatan, Merida, Mexico

Abstract: The etiology of obesity involves different factors, including the consumption of food with poor nutritional content. It is known that feeding animals with junk food promotes significant behavioral and molecular changes. In this regard, we have studied that rats under cafeteria diet protocol displayed chocolate preference as well as an enhancement in the expression of molecular marker, mitogen-activated protein kinase in hypothalamus. If chocolate preference is established, it is likely that neurochemical disruption could be linked with choosing this food item. Then, it is plausible to test the hypothesis that chocolate preference involve hedonic brain areas such as nucleus accumbens (AcbC) as well as endogenous molecules, including dopamine (DA). The aim of the present study was to describe whether D1 receptor antagonist drug (SCH23390) reduces chocolate-intake. To test the hypothesis, rats were fed with chocolate during 30 days. Next, animals were implanted with a cannulae aimed to the lateral hypothalamus as well as a microdialysis guide-cannula placed into the AcbC. After 7 days of recovery from surgical procedures, pharmacological challenges were carried out. Microdialysis samples were collected and analyzed to determine the levels of DA by HPLC means. All rats received either a microinjection of saline (control group) or SCH23390 (10µg/1µL) into the lateral hypothalamus and microdialysis samples were collected during 4h. It was found that microinjection of D1 receptor antagonist increased chocolate-intake. Despite this result, we found that endogenous levels of DA did not show statistical differences after the administration of SCH23390. Although it may be premature to suggest that blocking the activity of D1 receptor presumably placed into lateral hypothalamus, promotes chocolate-intake, preliminary data provides new insight regarding the potential mechanism of action of craving of junky food. This study was supported by Research Grant Program at Escuela de Medicina, Universidad Anáhuac Mayab given to E.M.-R.
Poster

524. Food Intake and Energy Balance: Monoamines and Other Regulators

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#/Poster#: 524.22/W39

Topic: E.07. Food Intake and Energy Balance

Support: Mishima Kaiun Memorial Foundation

JSPS Fellows15J07958

Title: Free access to running wheels normalizes hyperphagia and obesity in human growth hormone transgenic rats

Authors: *M. KOMATSUDA, K. YAMANOUCHI, T. MATSUWAKI, M. NISHIHARA; Vet. Physiology, The Univ. of Tokyo, Tokyo, Japan

Abstract: Obesity is a major health problem especially in developed countries. Increased food intake and decreased physical activity are considered as two major factors causing obesity. We previously produced human growth hormone transgenic (TG) rats, which are characterized by severe hyperphagia and obesity. Some previous studies show that voluntary exercise in a running wheel affect body weight not only by increasing energy consumption but also by decreasing food intake. Therefore, we examined whether voluntary running wheel exercise causes inhibition of hyperphagia and any alteration of body weight in TG rats. Male TG rats and their wild type littermates were maintained in cages with or without running wheels from 4 (immature) or 8 (mature) to 16 week of age. Food and water were provided ad libtim. Free access to running wheels completely abolished hyperphagia and obesity in TG rats, and this effect persisted until the end of the experiment as far as the running wheel was accessible. This effect was independent from ages that rats started voluntary running. Once those running wheels were locked and immobilized, hyperphagia of TG rats recurred immediately. Unexpectedly, though the running distances of TG rats were significantly less than those of wild type rats, it was sufficient to normalize their food consumption. Next, we conducted c-fos immunohistochemistry in brains of these rats to determine the neurons that are involved in normalizing hyperphagia by running wheels. C-fos positive neurons in the raphe nucleus and medial amygdaloid nucleus increased when rats were kept in running wheel accessible environment. However, activation of these neurons was canceled if running wheels were immobilized three hours before the sampling.
Our findings raise the possibility that rearing environment, which enables rats to access to a running wheel anytime they want, rather than the amounts or intensity of physical exercises, is more important for the maintenance of proper food intake and body weight. Furthermore, neurons in the raphe nucleus and medial amygdaloid nucleus could be relevant to normalizing hyperphagia in TG rats.


Poster
524. Food Intake and Energy Balance: Monoamines and Other Regulators

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 524.23/W40

Topic: E.07. Food Intake and Energy Balance

Title: Effect of frequent intake of steviol glycosides on the JAK2/STAT3 signaling pathway in the central nervous system of mice

Authors: *A. A. BARRIOS, J. A. ESTRADA, I. CONTRERAS;
Lab. de Neuroquímica, Univ. Autónoma Del Estado De México, Toluca, México

Abstract: Sweeteners are food additives that are commonly used worldwide. There are discrepancies in epidemiological studies evaluating their effect on body weight and appetite; this generates the need to further study the mechanism by which sweeteners could affect satiety and energy balance. This study’s main objective was to evaluate whether frequent intake of the steviol glycosides found in Stevia rebaudiana leaves could cause alterations in the JAK2/STAT3 signaling pathway, mediated by leptin, in the central nervous system of mice. For this purpose, two study groups composed of 14-week-old female BALB/C mice were established: a control group supplied with pure water, and a steviol glycosides group, which was supplemented with this sweetener in their daily water for 6 weeks. Mice were fed ad libitum and weight was measured weekly. The amount of food and drink intake was measured daily. After 6 weeks, body composition of mice was determined using bioelectrical impedance and total brain proteins were obtained, to determine the expression of total and phosphorylated JAK2 and STAT3 by Western Blot. Our preliminary results show that there was a higher consumption of food and water in the control group and also increased body weight, but mice supplemented with steviol glycosides had more adiposity. Regarding JAK2/STAT3, expression of pJAK2 was found to be diminished in the group supplemented with this sweetener, whereas we did not observe changes in the STAT3 proteins. Our results suggest that frequent intake of the steviol glycosides may cause
alterations in energy balance and appetite by altering the phosphorylation of JAK2. The alterations observed in adiposity in supplemented mice could be related to modifications in the activity of the thyrotropin-releasing hormone, which indirectly regulates adiposity in the organism and is controlled by the JAK2 signaling pathway.

Disclosures: A. A. Barrios: None. J. A. Estrada: None. I. Contreras: None.

Poster

524. Food Intake and Energy Balance: Monoamines and Other Regulators

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 524.24/W41

Topic: E.07. Food Intake and Energy Balance

Title: Pharmacological treatment as part of inpatient psychiatric treatment of anorexia nervosa patients; a clinical retrospective study

Authors: *A. H. LECKLIN;
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Abstract: Anorexia Nervosa (AN) is a serious psychiatric illness. Even though psychotropic drugs are not officially approved for adolescent AN, their use in recent years has increased and many clinicians prescribe these drugs for treatment of comorbid conditions of AN. This study examines the use of medications in adolescent AN inpatients treated in Kuopio University Hospital. A retrospective study of anorexia nervosa patients (n=82, 96% females) admitted to Department of Adolescent Psychiatry of the Kuopio University Hospital during January 1st 2002 and December 31st 2012 was conducted. Data from medical files were collected. The age of the patients at first admission was 15.6 years (range 11-17). On admission, mean body mass index (BMI) of the patients was 15.6 kg/m² (range 12.3-24.4 kg/m²). Every fifth patient had been vomiting, 62% were over-exercising, 9% had abused laxatives or other drugs for weight control. In every third of patients nutritional support was initiated with temporary nasogastric feeding. Psychiatric comorbidity was common: 65% had depression and every second (51%) anxiety. Approximately 15% of the patients showed obsessive-compulsive behavior and 15% were psychotic. One third of the patients (36%) had self-destructive behaviour. The average length of hospital stay was 88 days (range 1-612 days). Length of stay correlated with the decrease in the BMI. Psychopharmacological medications were prescribed to 84% of the patients. SSRIs and mirtazapine were the most common antidepressants while quetiapine and olanzapine were the most often used antipsychotics. Anxiolytic and sedative drugs had been prescribed to half of the patients. Psychopharmacological drugs were rather well tolerated except
for the antipsychotics that caused side effects in every second patient. At discharge, the psychosocial functioning of the patients had improved, their nutritional status was better and there were reductions in symptoms associated with eating disorders.

Disclosures: A.H. Lecklin: None.

Poster

524. Food Intake and Energy Balance: Monoamines and Other Regulators

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 524.25/W42

Topic: E.07. Food Intake and Energy Balance

Support: University of Guanajuato

Title: Decreased dopamine levels in overweight young women

Authors: *M. SOLIS-ORTIZ*, 1 C. SANDOVAL-SALA\-\-ZAR, 2 J. RAMIREZ-EMILIANO, 3 A. P. ROMERO-LÓPEZ; 1Inst. Invest Med, Univ. Guanajuato, Leon, Mexico; 2Enfermería y Obstetricia, Univ. de Guanajuato, Celaya, Mexico; 3Ciencias Medicas, Univ. de Guanajuato, Leon, Mexico

Abstract: Dopamine, a very powerful neurotransmitter, controls feeding of well-being. The consumption of carbohydrate binging stimulates the brain’s production of and utilization of dopamine and regulate Body Mass Index (BMI). Thus, in obese subjects dopamine deficiency may promote compensatory pathological eating to activate reward circuits. However, this relationship is remains unclear. The aim of this study was to evaluate the dopamine levels and consumption of kilocalories in overweight women. Twenty young women with a mean age of 31 years, with a BMI ≥ 25 Kg/m² and 20 young women with a mean age of 31 years, with a BMI ≤ 24.9 Kg/m² were evaluated. Dopamine levels in serum, consumption of kilocalories, micronutrients intake and percent body fat were measured and compared between groups. The overweight women compared with the normal weight group, showed decreased dopamine levels (p= 0.003), higher BMI (p= 0.001), higher percent body fat (p= 0.001), increased kilocalories, carbohydrates and lipids intake. This results suggesting that the low level of dopamine in serum may reflect the reward deficiency syndrome caused by a brain reward cascade dysfunction and resulting in abnormal craving behavior.

Intracranial EEG of hippocampal-amygdala dynamics during emotional memory discrimination

**Authors:** J. J. LIN\(^1\), R. F. STEVENSON\(^2\), S. L. LEAL\(^2\), J. ZHENG\(^1\), J. ROBERTS\(^2\), J. RILEY\(^1\), *M. A. YASSA*\(^3\);
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**Abstract:** Emotional arousal, mediated by the amygdala, is known to modulate episodic memories stored by the hippocampus, a region involved in pattern separation (the process by which similar experiences are stored using non-overlapping representations). Using high-resolution fMRI, we have previously shown that the DG/CA3 region of the hippocampus is engaged during accurate discrimination of highly similar items during performance on an emotional discrimination task. Furthermore, this effect was specific to negative compared to neutral items. However, the amygdala was modulated by emotion, regardless of memory performance (Leal et al., Hippocampus 2014). We suggested a potential mechanistic account for how emotional information is processed by the amygdala-hippocampal network. While high-resolution fMRI afforded us excellent spatial resolution, a higher temporal resolution is needed to characterize the dynamics of hippocampal-amygdala interactions. In the current study, we tested pre-surgical patients with epilepsy that had hippocampal and amygdala depth electrodes implanted to determine the locus of seizures. Using a high-resolution MRI anatomical template (.55mm isotropic), coupled with post-implantation individual subject MRI scans, we were able to determine the location of the depth electrodes within hippocampal and amygdala subregions. We analyzed power, coherence, and phase-amplitude coupling in the theta (4-8 Hz) and gamma (30-250 Hz) ranges during discrimination of similar emotional and neutral scenes. We found coordinated activity across the hippocampus and amygdala modulated by memory and emotion. Together with our high-resolution imaging findings, we are able to gain a better understanding of how the amygdala-hippocampal network subserves emotional episodic memory.

Poster

525. Hippocampus, Functional Networks, and Human Memory

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 525.02/W44

Topic: F.01. Human Cognition and Behavior

Support: NIMH R01 102392

NIA T32 AG027668

Title: High-resolution fMRI of hippocampal-amygdala dynamics during emotional memory discrimination in healthy aging and late-life depression

Authors: *S. L. LEAL1,2, J. A. NOCHE2, E. A. MURRAY2, M. A. YASSA2;
1Psychological & Brain Sci., Johns Hopkins Univ., Irvine, CA; 2Neurobio. & Behavior, Univ. of California, Irvine, Irvine, CA

Abstract: Episodic memory loss is a common facet of age-related cognitive decline as well as depression. Medial temporal lobe (MTL) structure and function are altered in both aging and depression, which includes loss of hippocampal volume due to subtle synaptic changes and a susceptibility to interference among similar experiences. Understanding the common and distinct neurobiological bases of age-related cognitive decline and late-life depression will allow us to more appropriately target interventions. We have developed an emotional discrimination task that is sensitive to subtle behavioral effects of emotional memory processing. This task explicitly manipulates stimulus interference (by varying scene similarity) as well as emotional content. Using high-resolution fMRI, we are able to ascribe the behavioral effects to interactions among hippocampal subfields and amygdala nuclei. Previously, we found that young adults with depressive symptoms showed a shift in hippocampal-amygdala activity, with increased amygdala activity and reduced DG/CA3 activity during discrimination of negative scenes (Leal et al., Hippocampus 2014). In the current study, we investigated activity within the hippocampal subfields and amygdala nuclei in older adults with and without depressive symptoms (age 60-90) as they performed the emotional discrimination task in the MRI scanner. We examined activity within hippocampal subfields and amygdala nuclei during accurate emotional discrimination. We find that depressive symptoms are associated with shifts in hippocampal-amygdala processing in...
These findings suggest that the MTL network is altered in aging and depression and that these two conditions may interact to confer added vulnerability in this network.

discrimination to be reduced in the FORWARD priming condition. Consistent with our prediction, we found that compared to the RANDOM condition, participants were more likely to false alarm to a similar lure object in the FORWARD priming condition, but not in the REVERSE condition. We discuss this result in the context of computational models of sequence memory as well as models of the DG/CA3 network as a multi-domain pattern separation/completion network.

**Disclosures:** J.M. Roberts: None. K.A. Kernodle: None. J.A. Noche: None. E.A. Murray: None. M.A. Yassa: None.

**Poster 525. Hippocampus, Functional Networks, and Human Memory**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 525.04/W46

**Topic:** F.01. Human Cognition and Behavior

**Support:** NIH 2R37NS21135

NIH NS060993 K23

**Title:** Network mechanism of amygdala and ventromedial prefrontal cortex during labeling of negative emotion

**Authors:** J. ZHENG¹, H. ERKOL¹, J. RILEY², G. GULSEN¹, K. ANDERSON³, R. KNIGHT³, *J. LIN²; ¹Dept. of Biomed. Engr., ²Dept. of Neurol., ³Dept. Neurolog. Surgery, ⁴Dept. of Neurobio. and Behavior, Univ. of California, Irvine, Irvine, CA; ⁵Helen Will Neurosci. Inst., ⁶Dept. of Psychology, Univ. of California, Berkeley, Berkeley, CA

**Abstract:** The ability to regulate negative emotions is an important adaptive skill that enables individuals to respond effectively to stress. The network controlling stress involves the amygdala and anatomical connections to ventromedial prefrontal cortex (VMPFC), anterior cingulate, and insula. During regulation of negative emotion, an inverse correlation between the amygdala and VMPFC has been observed in fMRI studies, leading to the hypothesis that VMPFC suppresses activation in the amygdala, in an effort to reduce emotional distress. To explore the electrophysiological mechanism regulating negative emotions, we recorded simultaneously from amygdala, hippocampus, VMPFC, anterior cingulate and insula using intracranial depth electrodes in 3 human subjects. During the task, each subject was asked to 1) label the emotion
by choosing one of two words on the right and left of the screen (angry, sad, afraid, happy, neutral) to describe the emotional face presented in the center; 2) match emotion by choosing one of the two emotional faces presented on the right and left side of the screen to match the emotional expression of a face presented in the center. The analysis focused on comparisons of spectral power and inter-trial phase coherence (ITC) between negative emotions (angry + sad + afraid) and neutral/happy emotions. Specifically, the extent of multimodal phase locking distribution across trials was quantified by phase bifurcation index (Busch et al., Journal of Neuroscience 2009) wherein a value of -1 denotes absence of synchronization across trials between the EEG signal and time locked stimulus, while a value of 1 indicates perfect phase locking in both conditions. The time frequency spectral analysis showed valence-selective increases in high-gamma activity (HG; 80-100Hz) for negative emotional trials relative to neutral/happy trials in the amygdala, hippocampus, VMPFC, and insula. In addition, phase bifurcation index value revealed that in the amygdala and insula, significant phase locking in the 4-8 Hz frequency band was present for both conditions (negative emotion and neutral/happy emotion). In contrast, the VMPFC showed selective phase locking in the low gamma range (50-60Hz) only for the negative emotional condition. These results provide evidence that the VMPFC employs gamma phase coherence as an oscillatory mechanism to discriminate negative emotions from neutral/happy emotion, whereas the amygdala and insula play a valence independent role in emotional processing.

precision scale along the anterior-posterior axis of the hippocampus. This body of work, however, has focused almost exclusively on spatial precision. Computational models of hippocampal function as well as empirical evidence from animal studies have suggested that the mechanisms underlying spatial and temporal memory processing are largely the same. We aimed to examine whether the precision of temporal memory is also represented differentially along the longitudinal axis. We developed a novel, naturalistic task to test the hypothesis that the anterior hippocampus supports memory for less precise temporal context, while the posterior hippocampus supports memory for more precise temporal context. During encoding, participants viewed a 30-minute video (sitcom), to which the participants had no prior exposure. During retrieval they were asked to place still frames from the video on a timeline (0 to 30 minutes). We assessed the precision of their judgments by calculating the temporal distance between the actual time at which the still frame occurred in the video and their indicated time. Based on this measure, we classified trials as low, medium, and high precision and we report activity within hippocampal subregions along the hippocampal axis. These results inform our understanding of the hippocampal division of labor and how the hippocampus represents temporal information.

Disclosures: M. Montchal: None. M.A. Yassa: None.

Poster

525. Hippocampus, Functional Networks, and Human Memory

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

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NSF Fellowship DGE-1232925

Roche/ARCS Foundation Fellowship

Title: Repeated study engages neocortex but disengages the hippocampus: Evidence for rapid systems consolidation?

Authors: *Z. REAGH*1,2, H. D. HO2, E. A. MURRAY2, M. A. YASSA2;

1Dept. of Neurobio. and Behavior, 2The Univ. of California, Irvine, Irvine, CA
Abstract: It is widely assumed that repeated study improves memory for all studied information. We recently proposed a theory that challenges this assumption on the basis of competing hippocampal memory traces (Yassa & Reagh, Frontiers in Behavioral Neuroscience 2013). One hypothesis arising from our theory is that repetition of an identical stimulus can induce highly similar but not perfectly overlapping memory traces, which can induce competitive memory interference and lead to a loss of detailed information during retrieval. We previously demonstrated behavioral evidence consistent with this phenomenon, where repeated study was found to boost target recognition but was detrimental to discrimination of similar lure items from those originally studied (Reagh & Yassa, Learning & Memory 2014). The present study replicated this behavioral outcome, and investigated the neural mechanisms underlying this tradeoff. Using whole-brain high-resolution fMRI (1.8mm isotropic), we found that repeated study induced a downward modulation of hippocampal activity, and an upward modulation of neocortical activity. Specifically, we report a repetition-mediated dynamic between the CA1 subfield of the hippocampus and anterior cingulate/medial prefrontal cortices, a relationship implicated in systems consolidation in animal models. We take these findings as evidence for competitive interference among overlapping memory traces, leading to more stable semantic memories at the expense of episodic details. We furthermore suggest that repetitions of a stimulus within a brief time frame may induce a rapid form of systems consolidation.


Poster

525. Hippocampus, Functional Networks, and Human Memory

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 525.07/X1

Topic: F.01. Human Cognition and Behavior

Support: NIMH R01 MH102392

Title: Remembering emotional gist and detail information: individual differences in age-related memory loss

Authors: *J. A. NOCHE*, S. L. LEAL, E. A. MURRAY, M. A. YASSA; 1Neurobio. and Behavior, Univ. of California, Irvine, Irvine, CA; 2Psychological and Brain Sci., Johns Hopkins Univ., Baltimore, MD

Abstract: Changes in memory performance are one hallmark of cognitive dysfunction in aging. However, it is unclear whether there are modulatory systems that may allow older adults to
compensate for these changes. Previously, we reported that older adults remembered both emotional gist and detail information, compared to young adults who only remembered more emotional gist information (Leal & Yassa, Behav Neurosci 2014, Leal et al., Neurobio of Learning & Memory 2014). It is unclear as to whether this general emotional memory enhancement in aging is compensatory or dysfunctional. A modified version of The Wechsler Memory Scale Logical Memory Subset was developed to assess and control emotional content and to examine gist and detail memory. Adults aged 60-85 were tested on their memory for the gist and detail information for three short stories (negative, neutral, and positive) after an immediate, 20-minute, and 1-week delay. A neuropsychological battery was administered, and subjects were split into aged impaired (AI) and aged unimpaired (AU) groups based on the Rey Auditory Verbal Learning Test (Rey, 1941). Overall, we found a bias for remembering negative gist and detail information, where the effect was largest after one week. The difference between immediate and 1-week delay performance was calculated (forgetting rate), where AI subjects showed a preservation of emotional gist and detail information and a selective forgetting of neutral information, while AU individuals did not exhibit this pattern of forgetting. However, AI subjects remembered positive gist and detail information much better than AU subjects, suggesting that the positivity bias in aging might be driven by individuals who show some memory loss. This may be a mechanism to compensate for a general memory deficit, where emotional information is prioritized at the expense of remembering neutral information.


Poster
525. Hippocampus, Functional Networks, and Human Memory

Location: Hall A

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Topic: F.01. Human Cognition and Behavior

Support: NIH Grant P50 AG05146

NIH Grant RO1 AG034613

Title: High-resolution fMRI of source memory and mnemonic discrimination

Authors: *R. STEVENSON, Z. M. REAGH, A. P. CHUN, E. A. MURRAY, M. A. YASSA;
UC Irvine, Irvine, CA
**Abstract:** Numerous studies have shown that the hippocampus and neocortex differentially contribute to item recognition and contextual source memory. However, the relationship between source memory and mnemonic discrimination of similar items is poorly understood. General item recognition is thought to be supported by the neocortex, whereas mnemonic discrimination is thought to be supported by the hippocampus, much like source memory. Using a well-validated object discrimination task modified to include a source memory component, previous work in our lab has shown that correct source memory judgements can occur in the absence of mnemonic discrimination (i.e. false alarms), indicating that these processes are behaviorally dissociable (Kim & Yassa, Hippocampus 2013). The present study used high-resolution fMRI to simultaneously assess hippocampal and cortical activity during mnemonic discrimination of similar items and source memory judgments for these items. We found that hippocampal and neocortical activity are differentially modulated by source memory and mnemonic discrimination, supporting the idea that these processes are indeed dissociable at a neural level. As source memory and mnemonic discrimination are thought to reflect recollection and pattern separation respectively, our data suggest distinct neural mechanisms for these computations.

**Disclosures:** R. Stevenson: None. Z.M. Reagh: None. A.P. Chun: None. E.A. Murray: None. M.A. Yassa: None.

**Poster**

**525. Hippocampus, Functional Networks, and Human Memory**

**Location:** Hall A

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**Topic:** F.01. Human Cognition and Behavior

**Support:** NSF BCS 1439267

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NIA T32 AG00096-31

**Title:** Hippocampus and medial prefrontal cortex show activity and functional connectivity during memory for sequences of events

**Authors:** *V. K. BOUCQUEY*, T. A. ALLEN, D. J. HUFFMAN, N. J. FORTIN, C. E. L. STARK;

Neurobio. and Behavior, Univ. of California, Irvine, CA
Abstract: Memory for the sequence of events is a critical component of episodic memory. However, the neurobiological substrates of this type of memory are not well understood, especially in humans. To address this, we acquired BOLD fMRI data in humans while performing our cross-species sequence memory task. The task tests memory for non-spatial sequences of events and shows cognitive parallels in rats and humans (Allen et al., 2014), allowing for multidisciplinary research. Subjects were presented with four different sequences of six distinct kaleidoscopic images in succession (e.g., Sequence “ABCDEF”). Subsequently, BOLD fMRI was acquired while subjects were presented images with all items in sequence (e.g., “ABCDEF”), or with one item out of sequence (e.g., “ABCEF”). Subjects initiated image presentations by pressing down a button. Images disappeared when the button was released, or after the decision threshold (1sec) was reached. If an image was “in sequence” the subjects were instructed to hold down the button until the decision threshold was reached (i.e. the image disappeared), if the image was “out of sequence” they were instructed to release the button before the decision threshold was reached. Successful sequence memory was demonstrated if subjects responded accurately to both “in sequence” and “out of sequence” items. Each subject accurately indicated items as “in sequence” and “out of sequence” at levels significantly greater than chance. We then completed a whole-brain analysis contrasting “in sequence” with “out of sequence” presentations. We found that the sequence task showed activation of the hippocampus (HC) and the medial prefrontal cortex (mPFC). In addition, we completed a functional connectivity analysis correlating the BOLD time-series within each of these regions (HC and mPFC) to the rest of the brain. Using the HC as the seed region, we found a reliable correlation of activity with the mPFC over the course of the task, and vice versa. Therefore, hippocampus and mPFC are active as well as likely interacting during memory for sequences of events. The results are consistent with previous research in rats that used a temporary inactivation approach to show the cross-species sequence task relies on the hippocampus and medial prefrontal cortex. Further, single-unit recordings in rats from the CA1 region of the hippocampus and prelimbic region of the medial prefrontal cortex show a dynamic and complementary series of changing representations that can solve the sequence task. The current results extend this work to humans and strongly suggest homologous underlying neural substrates in memory for sequences of events.


Poster

525. Hippocampus, Functional Networks, and Human Memory

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM
Title: Acute moderate exercise improves pattern separation in young adults

Authors: *K. SUWABE*, K. HYODO, K. BYUN, G. OCHI, M. YASSA, H. SOYA; 1Univ. of Tsukuba, Tsukuba City, Japan; 2Univ. of California-Irvine, Irvine, CA

Abstract: Many studies have revealed that exercise enhances hippocampal functions such as neurogenesis and spatial memory in animals, but there is less evidence for the critical component of episodic memory, pattern separation, associated with the dentate gyrus (DG) of the hippocampus. A recent study has suggested the beneficial effects of exercise on pattern separation in animals (Creer et al., PNAS, 2010), which should be possible considering the facilitatory role of acute exercise for the neuronal activities of the DG, as shown in our animal studies using a treadmill exercise model (Soya et al., BBRC, 2007). Also, considering that increased peripheral adrenalin levels modulate norepinephrine release in the hippocampus through the locus coeruleus (Miyashita et al., 2004), it is conceivable that increased sympathetic nerve activity (SNA) with exercise has an immediate impact on the DG and improves pattern separation ability. We thus postulated that this should be the case in humans. In the present study, twenty-one healthy young adults (mean age 20.9 ± 1.5 years, 8 females) participated in both of two experimental conditions: exercise and resting control. A mnemonic similarity task (MST) was performed after 10 minutes of moderate intensity exercise (50% Vo2peak) or rest (control). Salivary alpha-amylase (sAA) levels and the two-dimensional mood scale (TDMS) were measured at three time points (baseline, just after exercise, and just after encoding). Exercise enhanced discrimination of similar items (participants responded with ‘similar’ or ‘new’ for lure items) compared to rest (exercise: 62.0% vs. control: 58.5%). Analysis by item similarity showed significant main effects of both similarity and exercise. Traditional recognition memory performance (responses of ‘old’ for target items minus responses of ‘old’ for foil items) was unchanged with exercise. sAA and arousal levels measured with the TDMS increased after exercise (time point 2 minus baseline) compared to control. No correlations between sAA changes and MST performance changes or arousal changes and MST performance changes were found. These results support the hypothesis that acute moderate intensity exercise involving increased SNA may improve pattern separation in memory encoding, which may shed light on how exercise impacts memory functions associated with the hippocampus and its neuronal networks.

Disclosures: K. Suwabe: None. K. Hyodo: None. K. Byun: None. G. Ochi: None. M. Yassa: A. Employment/Salary (full or part-time); Univ. of California-Irvine. H. Soya: A. Employment/Salary (full or part-time); Univ. of Tsukuba.
Title: Cross-linguistic activation in hippocampal neurons of Spanish-English bilinguals

Authors: *E. K. HUSSEY*¹, K. CHRISTIANSON¹, P. N. STEINMETZ²;
¹Univ. of Illinois At Urbana-Champaign, Champaign, IL; ²Nakamoto Brain Res. Inst., Tempe, AZ

Abstract: The hippocampus declarative memory system (HDMS) supports the formation of long-term memory, including the acquisition, storage, and retrieval of explicit information (Squire, Stark, & Clark, 2004). These representations can be updated or consolidated over long periods of time to incorporate new sources of information (Eichenbaum & Cohen, 2001; Squire, Cohen & Nadel, 1984). To this end, the HDMS supports real-time language processing, especially during incremental sentence comprehension (Rubin et al., 2011) and referential communication (Duff & Brown-Schmidt, 2012). A strong test of the role of the HDMS in language involves bilingual processing; specifically, bilinguals dynamically activate both languages and flexibly switch between lexical entries while they process linguistic input (Bartolotti & Marian, 2012). To examine the neural mechanisms of this switch, we tested whether the firing of hippocampal neurons change in Spanish-English bilinguals under task conditions requiring flexible switching between their languages relative to within-language (non-switch) cases. We recorded single-unit activity in the hippocampus of Spanish-English epileptic patients as they completed a continuous recognition memory task containing words in both languages. Participants were instructed to judge semantic repetitions over the course of the experimental session; half of the targets repeated in the same language (non-switch trials; e.g., dog appearing after dog) and half repeated in the opposite language (switch trials; e.g., perro appearing after dog). Behaviorally, participants were less accurate and slower to respond to switch relative to non-switch trials (Accuracy: t=4.54, p<0.001; Correct Frequency-Adjusted Response Time: t=2.09, p=0.04). Neurally, we observed increased average hippocampal firing for correct targets compared to correct non-targets, replicating Wixed et al., 2014 (t=−3.64, p<0.001). Of the targets, switch trials elicited greater firing relative to non-switch items in the
right hippocampus ($t=-3.61, p=0.003$). Interestingly, this effect reversed in left hippocampus ($t=2.88, p=0.02$), such that switch trials were associated with less firing compared to non-switch cases. This pattern provides support for lateralized repetition-suppression, perhaps due to a left hemispheric bias for semantic processing (Martin, 1999). Finally, we provide the first evidence that flexible cross-linguistic lexical access constitutes another case of HDMS involvement in the language domain.

**Disclosures:** E.K. Hussey: None. K. Christianson: None. P.N. Steinmetz: None.

**Poster**

**525. Hippocampus, Functional Networks, and Human Memory**

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**Topic:** F.01. Human Cognition and Behavior

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**CIHR 129855**

**Title:** Hippocampal contributions to configural probabilistic learning

**Authors:** *K. D. DUNCAN*, B. B. DOLL, N. D. DAW, D. SHOHAMY;  
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**Abstract:** Extensive research has characterized the mechanisms by which the striatum supports stimulus-response learning. A crucial, yet overlooked, component of this learning is identifying the relevant ‘stimulus’ amongst the array of features that comprise an experience. This is challenging, in part, because it is not always clear what makes a “stimulus”: a stimulus can be a singular individual element, or, it can be a full configuration of features that comprise an experience. We hypothesized that the neural mechanisms that support learning will depend on how the stimulus is defined. Specifically, that the hippocampus, well known for its role in encoding stimulus-stimulus associations, will be involved in stimulus-response learning when the stimulus itself is a configuration of cues. To test this hypothesis, we had participants perform a probabilistic classification task (a variant of the "weather prediction" task) while undergoing fMRI scanning (n=26). Importantly, in our task, abstract cues were independently associated with weather outcomes but were never presented alone and were combined into a small number
of combinations. This design allowed participants to learn either by associating individual cues with outcomes (elemental learning), or by associating the configuration of cues with outcomes (configural learning). We used a reinforcement-learning model to quantify the influence that recent experiences with individual cues and cue-combinations held over participants' choices. This model allowed us to identify (1) individual differences in elemental vs. configural learning and (2) the extent to which each choice reflected elemental vs. configural learning. We found that hippocampal BOLD activity was stronger when people made choices that reflected recent history with cue-combinations, as opposed to individual cues, and that this relationship was most evident in participants whose choices reflected configural learning. Additionally, participants who engaged in more configural learning showed greater functional connectivity between the hippocampus and nucleus accumbens during learning. Furthermore, feedback-related responses in the nucleus accumbens were more correlated with hippocampal activations during choice in participants who engaged in configural as compared to elemental learning. By contrast, people who engaged in elemental learning showed greater functional connectivity between the nucleus accumbens and dorsal striatum than those who engaged in configural learning. Together, these results suggest that the hippocampus may contribute to probabilistic learning by forming configural representations of task states.


Poster

525. Hippocampus, Functional Networks, and Human Memory

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 525.13/X7

Topic: F.01. Human Cognition and Behavior

Support: NIH Grant MH0849

NIH Grant DA036361

Title: First-episode schizophrenia is associated with disrupted network connectivity across separable hippocampal subsystems

Authors: *V. P. MURTY1, A. TOMPARY2, B. ZENG4, J. WANG4, D. GOFF5, L. DAVACHI3; 1NYU, New York, NY; 2New York Univ., New York City, NY; 3New York Univ., New York City, NY; 4Shanghai Mental Hlth. Ctr., Shanghai, China; 5Nathan Kline Inst., New York City, NY
Abstract: Hippocampal neurophysiology and network connectivity have been critically implicated in the etiology and symptomatology of schizophrenia. The hippocampus is a complex structure consisting of many subsystems, which may differentially interact with discrete cortical targets to contribute to cognition. While few studies have investigated hippocampal networks in schizophrenia, relatively little is known as to how different subsystems of the hippocampus are implicated in this disorder. In the current study, we compared network connectivity of hippocampal subsystems across 29 first-episode schizophrenic patients and 29 matched controls. Data were collected as part of a larger cohort collected at the Shanghai Mental Health Center investigating biomarkers of anti-psychotic treatment in first-episodic schizophrenia. Network connectivity analysis was implemented using a dual-regression approach on resting state fMRI. The hippocampus was functionally segmented into 8 sub-regions using a data-driven approach, i.e. independent spatiotemporal profiles. We found that only four of the eight subsystems showed significant differences in network connectivity across groups: two separable but overlapping regions in right anterior hippocampus, a region of left anterior hippocampus, and a region of left posterior hippocampus (p<0.05 whole-brain corrected, bonferroni corrected across subsystems). In general, connectivity profiles of these regions showed greater connectivity with lateral prefrontal regions in controls versus patients, and greater connectivity with thalamic, posterior midline, and ventral visual regions in the patients versus controls. Critically, all schizophrenia patients were naïve to antipsychotic drugs suggesting that these connectivity differences reflect etiology rather than medication. In general, these findings support a model by which connectivity between cortical targets and only particular subset of hippocampal regions are implicated in schizophrenia.


Poster

525. Hippocampus, Functional Networks, and Human Memory

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Topic: F.01. Human Cognition and Behavior

Support: NIH Grant AG029411

Title: Category specific modulation of resting state networks predicts memory performance

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Abstract: Memory consolidation theories predict that coordinated activity between the hippocampus and cerebral cortex is responsible for the storage of a long-term memory trace and that this process is mediated by the re-activation of regions during rest that were active during encoding (reviewed in Tambini, Ketz, & Davachi, 2010). Reactivation mediated memory consolidation occurs during periods of wakeful rest and the associated increase in hippocampal-cortical connectivity has been related to individual differences in associative memory performance (Tambini et al., 2010). A long history of research has supported the existence of specialized cortical regions in the fusiform gyrus and perirhinal cortex that are engaged during the perception and recognition of faces but not other visual stimuli (reviewed in Collins & Olson, 2014). Similar category specificity has been observed in a region of the parahippocampal gyrus for the visual encoding of places (Epstein & Kanwisher, 1998). Reactivation theories predict that face- and place- memory encoding should modulate connectivity within these category selective regions and that this modulation should predict behavior. The goal of the current study was to test this hypothesis. Participants completed a baseline resting-state scan followed by two encoding tasks in which they responded to images of faces or places. Each encoding task was followed by an additional resting state scan and recognition memory for the learned stimuli was assessed outside of the scanner. Face-selective regions of interest (ROIs) were defined in the fusiform gyrus (FFA) and perirhinal cortex (PRC), and a place-selective ROI was defined in the parahippocampal gyrus (PPA). Additional ROIs were defined in the left and right hippocampi that maximally responded to stimuli that were later remembered regardless of category. A significant increase in connectivity was observed between the PPA and left hippocampus following the place but not face-encoding task. After the face-encoding task connectivity was significantly decreased between the PPA and face processing regions (PRC and FFA) as well as the right hippocampus. A whole brain linear regression revealed that the modulation of connectivity between the PPA and the posterior parietal cortex after place encoding predicted individual differences in the recognition of places (but not faces). Individual differences in the recognition of faces (but not places) were predicted by modulated connectivity between the FFA and PRC ROIs after face encoding. Our results thus provide novel evidence for category specificity in the neural mechanisms supporting memory consolidation.

Disclosures: J.A. Collins: None. B.C. Dickerson: None.

Poster

525. Hippocampus, Functional Networks, and Human Memory

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 525.15/X9
**Title:** Proactive brain network dynamics predicts episodic memory in children

**Authors:** *S. Qin*¹, S. Prathap², J. Kochalka², S. Ryali², V. Menon²;  
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**Abstract:** It is proposed that the human brain is proactive in that it modulates subsequent information processing (Bar, 2007). But whether and how the brain’s intrinsic functional organization can proactively impact the efficiency of subsequent learning and memory is not known. Previous studies have only focused on the neurobiological processes of learning and memory in adults at the acquisition and retrieval stages, our understanding of these processes in children is poor. Even in adults, it is unknown how intrinsic brain activity and network configuration contributes to the formation of new memories. To address these questions we combined resting-state and task-related fMRI prior to and during memory encoding in 24 young children (aged 8-14). Brain imaging data was acquired on a 3T GE Signa scanner using a T2*-weighted spiral in-out sequence to gain signal sensitivity and mitigate signal drop-outs. Using a standard subsequent memory paradigm we first identified brain regions (nodes) involved in successful episodic memory formation. We then used a novel Bayesian hidden Markov models (HMM) (Ji & Carin, 2006) to identify dynamic functional networks (DFNs) between these nodes, during rest prior to memory encoding, and its links to subsequent memory performance. Three findings are noteworthy. First, we observed a distributed network of 20 regions (nodes) in the temporal, medial temporal, parietal, and frontal lobes involved in successful episodic memory formation in children, with the left anterior hippocampal activation predictive of individual differences in children’s memory performance. Next, we found three major communities of DFNs of these 20 nodes in the resting state prior to memory encoding. Critically, one of the more frequently occurring DFN that comprising the d medial temporal lobe, prefrontal and temporal-parietal cortices negatively predicted subsequent memory performance. These results suggest that proactive brain network dynamics can impact subsequent memory performance. By characterizing proactive brain network dynamics and their links to subsequent episodic memory, our findings provide new insights into how intrinsic brain network organization can influence the formation of new memories. More broadly, our study highlights the importance of proactive brain dynamics in support of children’s learning and memory.

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S. Qin: A. Employment/Salary (full or part-time); Stanford University, NIH, BNU. S. Prathap: None. J. Kochalka: None. S. Ryali: None. V. Menon: None.
Poster

525. Hippocampus, Functional Networks, and Human Memory

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UCLA Academic Senate grant

Title: Deactivation of brain regions associated with subsequent forgetting predicts effects of value on recognition memory in both young and older adults

Authors: *M. S. COHEN*¹,², B. J. KNOWLTON², A. D. CASTEL², J. RISSMAN²;
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Abstract: There has been growing interest in the phenomenon of “negative subsequent memory” (NSM) effects. These effects, characterized by greater fMRI activity during the encoding of items that are subsequently forgotten relative to those that are subsequently remembered, are thought to reflect changes in internally directed cognition detrimental to learning. Notably, NSM effects tend to be reduced or eliminated in older adults. Presently, we were interested in whether an item’s value (i.e., how many points could be earned by recalling that item later) would impact activity in NSM regions during encoding, and whether such value-related changes in activity would affect memory independently from changes in positive subsequent memory (PSM) regions. We were also interested in whether young and older adults would differ for either type of effect. 19 young and 21 older adult participants studied a series of word lists in an fMRI scanner, one word at a time. Each word was preceded by a point value indicating how important the word would be to remember. Memory was initially assessed using a free recall test, but shortly after the scan session, participants were tested again using a recognition test with a 1-6 confidence scale. To better characterize subsequent memory effects in our data, we defined two networks of interest based on a meta-analysis (Kim, 2011) of PSM and NSM effects,
respectively. We found reduced activity in the NSM network during encoding of high- relative to low-value items (significant in young; marginal in old). In addition, in both age groups, adding value-related differences in brain activity in NSM regions to a regression analysis explains significantly more of the value-related difference in recognition confidence than value effects in PSM regions alone. Although the value-related difference in activity in NSM and PSM networks tends to be positively correlated, the beta coefficients are significant in opposite directions. Value-related differences in NSM regions, but not PSM regions, still have significantly negative beta weights when recognition performance scores are re-computed using only those items that were not recalled during the initial free recall test. Together, these results demonstrate that activity in NSM regions is affected by one’s motivation to learn individual items. Moreover, these effects appear to be dissociable from value effects observed in PSM regions, suggesting that activity within these networks may be independently regulated. Thus, both young and older adults appear to down-regulate neural activity that is detrimental to encoding in order to enhance memory for valuable items.

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**Poster**

**525. Hippocampus, Functional Networks, and Human Memory**

**Location:** Hall A

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**Topic:** F.01. Human Cognition and Behavior

**Support:** NSERC RGPIN-2015-03838

**Title:** Combined physical exercise and cognitive training enhances hippocampal-dependent memory in young adults

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**Abstract:** There is an established link between exercise, neurogenesis, and cognition. Most of this research has focused on non-human animal models, with little known about the effects of exercise on cognition in younger adults. Both physical exercise and cognitive training independently induce hippocampal neurogenesis in animals, suggesting that these different forms of training may work through synergistic neural pathways to benefit memory in younger adults. The present study examined the effects of physical exercise and cognitive training on
hippocampal-mediated memory processes in younger adults, to determine whether combined training yields synergistic benefits. Sixty-six sedentary young adults (age range 18-30 years) were randomly assigned to one of four groups: 1) exercise training group, 2) cognitive training group, 3) combined exercise and cognitive training group, or 4) control group. Memory performance was assessed before and after the six-week intervention on the Pattern Separation task, which targets the dentate gyrus and is associated with hippocampal neurogenesis. Serum brain-derived neurotrophic factor (BDNF) and insulin-like growth factor 1 (IGF-1), factors that support neurogenesis, neuronal survival and function, were also assessed. Combining exercise and cognitive training led to the greatest increase in memory performance requiring pattern separation, but this was accompanied by a corresponding decrease in memory performance requiring pattern completion. BDNF and IGF-1 were not associated with this change in memory performance but were associated with the individual’s response to the exercise training, such that high responders to exercise had greater BDNF and IGF-1 than low responders to exercise. The results suggest that exercise and cognitive training may work through synergistic pathways to bias hippocampal function towards pattern separation. However, BDNF and IGF-1 may not be mediating this change in memory function.

Disclosures: J.J. Heisz: None. I.B. Clark: None. M. Fahnestock: None.

Poster

525. Hippocampus, Functional Networks, and Human Memory

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Topic: F.01. Human Cognition and Behavior

Support: NIH Grant R01-AG19731 to RC

Title: Hippocampal contributions to the large-scale episodic memory network predict vivid visual memories

Authors: *M. STANLEY, B. GEIB, E. WING, R. CABEZA; Duke Univ., Durham, NC

Abstract: Multivariate functional connectivity analyses of neuroimaging data using graph theory have revealed the importance of complex, distributed interactions between disparate yet interdependent brain regions for successful cognitive functioning. For the vivid, detailed retrieval of recently experienced events, the hippocampus in particular is thought to play an integral role in facilitating the flow of information throughout the network and integrating specific
information derived from different brain regions. After constructing functional brain networks derived from the beta series of a slow event-related fMRI investigation of visual memory retrieval, we examined differences hippocampal topological properties identified between vivid and dim memories of previously encoded items. Vivid memories for retrieved items were associated with more efficient communication for the hippocampus throughout the entire brain network, as well as with a more central, influential role for the hippocampus in network integration. More specifically, vivid memory for retrieved items were associated with the hippocampus (1) participating in more efficient communication with the rest of the network, (2) exhibiting an increase in connectivity strength with diverse brain regions, (3) displaying stronger connectivity with more central, influential nodes in the network, and (4) massively reorganizing its set of direct connections with the rest of the network. These results underscore the potential of multivariate brain connectivity research for providing valuable new insights into the neural bases of memory processes that extend prior work focused on localizing memory processes to particular cortical areas using univariate methodologies.

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healthy subjects were shown 190 b&w objects presented with a blue or yellow frame indicating requirement of a Go button press or NoGo response (with equal number of Go and NoGo trials to avoid "oddball" effects on memory encoding). A recognition test was conducted 1hr later. All encoded images (without frame) were presented plus 190 new images, with subjects indicating whether they remembered (R), were familiar with (K) or did not remember (forgotten, F) the image from the encoding phase. In Exp 1, image presentation time was 1s in both phases, with variable ISI. Exp 2 was identical except that subjects were financially rewarded for responding as fast as they could, and financially penalised for commission errors. Exp 3 was identical to Exp 1 except that stimuli were presented for 250ms. Exp 4 was a replication of Exp 3 in the context of functional MRI scanning. Following pre-processing, using SPM8, memory encoding-related activity was analysed in a GLM comprising regressors modeling encoding responses for subsequent R, K and F trials separately for Go and NoGo. Ensuing contrast images for main effects of Go vs NoGo and R vs F, and the interaction were entered into one-sample t-tests across all subjects. Our behavioral studies consistently reveal better remember accuracy for stimuli paired with Go responses. An interaction between motor response and subsequent memory is observed in an area of dorsal pons consistent with the locus coeruleus (LC). Electrophysiological recordings in non-human primates show phasic LC activity during motor acts2. Given that LC activity leads to the release of noradrenaline throughout the brain, and that noradrenaline enhances memory3, we suggest that motor modulation of memory is mediated by the noradrenergic system.

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Poster

525. Hippocampus, Functional Networks, and Human Memory
Title: The effect of sedation on reconsolidation of emotional episodic memory in humans

Authors: *A. I. GALARZA VALLEJO*, M. C. W. KROES, E. REY DÍAZ-RUBIO, M. ACEDO, G. FERNANDEZ, B. STRANGE;

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Abstract: There is increasing evidence that memories are not stable but reflect a dynamic process. Following reactivation, memories can become labile and must undergo reconsolidation in order to persist. Disrupting memory reconsolidation may be a promising tool for treating psychiatric diseases. We recently showed that an electroconvulsive therapy (ECT) procedure impairs reconsolidation in unipolar depression patients. Specifically, reactivating memory for an emotional story immediately before ECT impaired subsequent recognition, relative to a non-reactivated story, when tested after 24h but not if tested immediately after ECT. One limitation of this study, however, is that it could not determine which component of the ECT - the general anesthetic (GA), the electric current applied to the cranium, or the subsequent seizure - produced reconsolidation impairment. In the current study, we therefore tested the effect of propofol (a short-acting hypnotic used for GA induction and maintenance) on reconsolidation in psychiatric/neurologically normal individuals undergoing brief GA for a routine procedure (endoscopy). Our emotional memory paradigm included two groups. Both groups encoded two emotional stories (identical to those used previously) one week prior to endoscopy. Each emotional story comprises 3 phases, 1 and 3 being neutral and phase 2 emotionally aversive. For both groups, reactivation of one of the stories (by presenting the first slide of one story only) was conducted in the endoscopy room 1-2 min before receiving propofol. Following endoscopy, both groups underwent a multiple choice memory test, but at different delays: group A after 24 hours and group B after ~90 minutes. Propofol impaired reconsolidation of memory for the emotional phase of the story (Phase 2) in Group A only. A Group (A, B) by Reactivated (yes, no) by Phase (1,2,3) rmANOVA reveals a significant 3-way interaction for the quadratic contrast $F_{1,16}=4.73$, $P=0.045$. Our data therefore demonstrate that a routine anesthetic procedure can impair reconsolidation selectively for emotional episodic memories.

Poster

525. Hippocampus, Functional Networks, and Human Memory

Location: Hall A

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Topic: F.01. Human Cognition and Behavior

Support: MRC

Wellcome Trust

Department of Health NIHR UCLH/UCL Biomedical Research Centre

Title: Hippocampal theta during memory guided virtual navigation in human intracranial EEG

Authors: *D. BUSH*1, J. A. BISBY1, C. M. BIRD2, S. GOLLWITZER3, R. RODINOV4, C. SCOTT5, B. DIEHL4, A. W. MCEVOY4, M. C. WALKER4, N. BURGESS1;

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Abstract: Theta frequency oscillations are prominent in the rodent hippocampal local field potential (LFP) during movement, and are typically in the range of 6-10Hz. Theta oscillations are also associated with human spatial memory function, typically in the range of 3-7Hz. However, the exact relationship between human theta oscillations, movement and spatial memory function is currently unclear. We examined intracranial EEG recordings from depth electrodes located in the hippocampi of twelve pre-surgical epilepsy patients performing a self-paced virtual reality navigation and spatial memory task. In this task, participants were asked to navigate towards, and encode the location of, various visible objects within a single environment. Participants were subsequently cued with the image of a single object, then placed back in the environment and
asked to navigate to the remembered location of that object. We found that power in the higher frequency (6-10Hz) theta band increased significantly during a 1s period around virtual movement onset compared to 1s stationary periods, consistent with MEG findings in a similar task (Kaplan et al., 2012). Moreover, 6-10Hz theta power during this 1s movement onset period correlated with subsequent memory performance. An increase in lower frequency (3-6Hz) theta power was also observed around movement onset, but did not correlate with subsequent memory performance. Next, we identified a more sustained increase in broadband ~3-16Hz oscillatory power during the 3s cue period, but only 6-10Hz theta power during this 3s period correlated with subsequent memory performance. These findings suggest that human hippocampal theta oscillations in the higher (6-10Hz) band are associated with both virtual movement and the accuracy of spatial memory function. Kaplan R, Doeller CF, Barnes GR, Litvak V, Duzel E, Bandettini PA, Burgess N (2012) Movement-related theta rhythm in humans: coordinating self-directed hippocampal learning. PLoS Biology e1001267

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Poster

526. Human Memory Retrieval and Reactivation

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

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Topic: F.01. Human Cognition and Behavior

Support: NIH Grant 5 T32 DC 9399-5

Title: Reactivation and anterograde disruption of recently encoded memories: A variant of reconsolidation

Authors: *R. B. BAUDO, B. A. WRIGHT;
Communication Sci. and Disorders, Northwestern Univ., Evanston, IL

Abstract: During learning, memories are transformed from a fragile to a stable state through a process of consolidation. There is growing evidence that when consolidated memories are accessed they re-enter a state of instability, during which they can be modified, and then must be consolidated again. This cycle of reactivation and reconsolidation is revealed by reports that
consolidated memories are susceptible to retrograde interference following a reminder of the memory, even when the reminder occurs weeks to months after the initial training. Here we show behavioral evidence in humans of a mechanism similar to reconsolidation, but in which reactivated memories are susceptible to anterograde interference only within 24 hours of the initial training. Using a perceptual-learning paradigm, we first identified a case in which learning on a target task was disrupted in the anterograde, but not retrograde, direction. Learning across days on interaural-level-difference discrimination (L) (the target task) was disrupted by practicing interaural-time-difference discrimination (T) (the non-target task) before (TL; n=11), but not after (LT; n=10), daily practice on the target task. We then used a paradigm akin to that used to test for reconsolidation. Listeners practiced a bout of the target task in isolation (L) and 30 minutes later practiced the task order that elicited anterograde interference (TL) (L30minT; n=14). This regimen yielded no improvement on the target task the day after training, suggesting that the initial bout of the target task was reactivated by the reminder and that both bouts were disrupted by the anterograde interferer. Learning returned when the reminder was removed (L30minT, n=10), confirming that the lack of learning in the L30minTL case was not due to retrograde interference of the non-target task (T) on the initial bout of the target task (L), and that the disruption of learning required the target-task reminder. Increasing the time between the initial and reminder bouts from 30 minutes to 24 hours (L24hrT; n=14) yielded improvement on the target task from the initial bout to the test 48 hours later, but this learning occurred between the initial bout and the reminder, and not between the reminder and the test. Thus, after 24 hours, while the initial bout was no longer susceptible to anterograde interference, new bouts of training on the target task were. These data suggest that during an early stage of memory formation, recently acquired memories can be reactivated, but are susceptible to anterograde interference, while older memories are protected from such disruption.

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Poster

526. Human Memory Retrieval and Reactivation

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Support: MacArthur Foundation's Research Network on Law and Neuroscience

NDSEG

SGF
Title: The effects of anticipatory stress on the neural correlates of associative memory retrieval

Authors: *S. A. GAGNON, A. D. WAGNER; Psychology, Stanford, Stanford, CA

Abstract: As we go through the world, we continually retrieve information about prior experiences to guide decisions and actions in the present. The process of recollecting specific, associative details about past events involves mechanisms supported by the medial temporal lobe (MTL) and frontoparietal cortical areas. Stress is known to alter MTL processing, and acute stress may also impair function in frontoparietal networks critical for controlled processing. Here, we investigated whether acute anticipatory stress influences memory retrieval processes. Stress was operationalized as the threat of an unpredictable shock. We hypothesized that associative retrieval performance is vulnerable to interference under conditions of stress relative to safety, and that this occurs in part through altering the engagement of the MTL and frontoparietal controlled processes. During encoding, words were paired with either an indoor or outdoor scene while subjects were in a neutral state. At retrieval 24 hours later, subjects were placed into a stress group or control group, and viewed old and new word cues while we recorded physiological measures (electrodermal activity, heart rate, salivary cortisol) and neural activity (via fMRI). If subjects recognized the word as old, they were further asked to recollect the associated scene. Critically, stress subjects were tasked with retrieving information under conditions of stress (threat of shock) or safety. Results revealed that stress during retrieval modulated physiological activity, affected behavioral performance, and influenced retrieval-related neural activity.

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Poster

526. Human Memory Retrieval and Reactivation

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Title: Structural and functional contributions to context-dependent relational memory

Authors: *H. SCHWARB*¹, C. L. JOHNSON², J. L. HOLTROP², J. X. WANG³, P. D. WATSON², J. L. VOSS³, N. J. COHEN²;
¹Univ. of Illinois, Urbana, IL; ²Univ. of Illinois Urbana-Champaign, Urbana, IL; ³Northwestern Univ., Chicago, IL

Abstract: Separate contributions of the medial temporal lobe (MTL) and prefrontal cortex (PFC) have been frequently implicated in support of memory function. Research suggests that the MTL is critically involved in forming relational memory representations and the PFC is involved in extracting and using more abstract associative rules. We present data from a novel relational memory task in which participants learn that individuals belong in a single room in each of two buildings. Room assignment is consistent with an underlying contextual-rule structure in which male and females are assigned to opposite sides of a building and the side-assignment switches between buildings. The buildings are dissociable by color and each building serves as a separate context. Diffusion Tensor Imaging (DTI) data was collected from 20 healthy young adults and behavioral performance was correlated with two tracts involved in memory processing: the fornix/fimbria and the uncinate fasciculus. The fornix/fimbria is a white matter tract primarily within the MTL that connects the hippocampus to the thalamus through the mammillary bodies and has previously been associated with memory recall. The uncinate fasciculus is a white matter tract that connects the anterior temporal lobe to the inferior frontal gyrus through the MTL and has been implicated in episodic memory. Our data reveal that fractional anisotropy (FA) of the fornix/fimbria is related to successful learning of specific room associations, while uncinate fasciculus FA is related to successful contextual-rule abstraction and use. These data suggest that the fornix and uncinate fasciculus play distinct roles that contribute to successful memory performance on this novel relational memory task. Functional magnetic resonance imaging (fMRI) data in a separate group of 20 healthy young adults are consistent with the DTI data. Successfully learning of specific room associations activated the hippocampus while successful contextual-rule abstraction and use was associated with activity in the anterior frontal lobe. Together these data suggest that MTL and PFC structures exert separable contributions to accurate relational memory performance in this task.


Poster

526. Human Memory Retrieval and Reactivation

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM
Title: Evidence for the representation of context in human parahippocampal cortex and retrosplenial cortex

Authors: *D. J. HUFFMAN, C. E. L. STARK;
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Abstract: Decades of research have established that the structures in the medial temporal lobe play an essential role in the representation of contextual information, particularly the representation of spatial information. A recent rodent study extended these findings by providing evidence that the hippocampus contains hierarchically-organized conjunctive representations of items bound to positions within particular contexts (McKenzie et al., Neuron, 2014). We sought to apply a similar approach in humans using functional magnetic resonance imaging (fMRI). Similar to the rodent task, our task contained 8 events which consisted of two contexts and two sets of object pairs. Participants learned event-location associations through trial-and-error learning. The correct location was unique based on: 1) the context, 2) the object pair, 3) the order in which the objects were presented. Thus, participants were required to distinguish between overlapping associative memories. Participants learned the associations the day prior to completing a fMRI scan session. Several lines of evidence suggest that parahippocampal cortex (PHC), retrosplenial cortex (RSC), and posterior cingulate cortex (PCC) are part of a network of cortical regions involved in the representation of contextual information (cf. Ranganath and Ritchey, Nat Rev Neurosci, 2012). Using multivariate pattern analysis of fMRI data, we have previously shown that PHC and RSC/PCC contained similar category information (faces vs scenes) on a trial-by-trial basis (Huffman and Stark, Hippocampus, 2014). In the current study, multivariate pattern analysis revealed that both PHC and RSC/PCC contain context-specific information. Specifically, patterns of activity elicited in response to events that shared the same context were more similar to each other than to events that took place in the other context. Additionally, PHC and RSC/PCC contained similar representations of context on a trial-by-trial basis, supporting the theory that PHC and RSC/PCC are part of a network of regions involved in the representation of contextual information. Further, we found that patterns of activity in RSC/PCC were more similar in response to events that shared the same context and the same items than in response to events that shared the same context but not the same items, thus providing evidence for conjunctive representations within RSC/PCC. The current results extend previous findings, which have largely been based on category-level differences, to the realm of the representation of individual contexts.
Disclosures: D.J. Huffman: None. C.E.L. Stark: None.

Poster

526. Human Memory Retrieval and Reactivation

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

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Topic: F.01. Human Cognition and Behavior

Support: McDonnell Foundation Grant #220020387
NIDCD R01 DC011755
NIMH R01 MH062500
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NIH R01 MH100121

Title: Impairments in associative inference following damage to the ventromedial prefrontal cortex

Authors: *K. SPALDING*¹, M. L. SCHLICHTING², D. ZEITHAMOVA³, A. R. PRESTON², M. C. DUFF³, D. TRANEL³, D. E. WARREN¹;
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Abstract: Learning in one situation and later applying that knowledge during new experiences is a critical cognitive ability that helps organisms adapt to changing environments, but the neural bases of this adaptive inferential behavior are not fully understood. Laboratory tests of associative inference in humans have examined memory performance for overlapping pairs of items (e.g., AB, BC) and for non-studied inference pairs that shared a common associate (i.e., AC). Using this paradigm, functional neuroimaging studies have shown that increased activation and functional connectivity of ventromedial prefrontal cortex (vmPFC) and hippocampus during encoding of overlapping pairs are associated with superior performance during subsequent inference (Zeithamova, Dominick, & Preston, 2012). In the current study, we used a neuropsychological approach to test the necessity of vmPFC for normal associative inference. Specifically, we tested participants with focal brain damage including vmPFC (N=6; non-amnesic) and demographically-matched healthy normal comparison (NC) participants (N=12) using the paradigm described in Zeithamova et al. 2012. Based on the earlier neuroimaging
findings and also on neuropsychological data suggesting a special role for vmPFC in associative or schematic memory (Warren, Jones, Duff, & Tranel, 2014), we predicted that the vmPFC group would show impairments in the ability to draw associative inferences based on memory despite intact memory for studied items. Participants studied overlapping pairs of items (AB and BC). Participants then completed a three-alternative forced-choice recognition task for studied (AB and BC) and corresponding inferential relationships (AC). Interestingly, despite normal memory on standard neuropsychological tests, the vmPFC group showed a significant impairment for studied pairs. Moreover, as predicted, the vmPFC group was significantly impaired when tested with items requiring associative inference based on memory for studied pairs, even when controlling for the memory impairment for studied pairs. These findings suggest that vmPFC is necessary for memory processes that support learning of overlapping events and novel associative inference. More broadly, these results provide insight into the neural bases of our adaptive ability to integrate existing knowledge with novel circumstances and reinforce a role for the vmPFC in such memory integration.


Poster
526. Human Memory Retrieval and Reactivation

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Topic: F.01. Human Cognition and Behavior

Support: NIMH Grant R01MH072966

Title: The core recollection network dissociates as a function of phenomenal and objective recollection

Authors: *P. P. THAKRAL, S. S. YU, M. D. RUGG;
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Abstract: It has been reported that hippocampal recollection effects are sensitive not to whether a test item elicits a phenomenal sense of recollection, but to the amount or fidelity of the contextual information retrieved about the study episode. In a previous study (Yu, S.S., Johnson, J.D., & Rugg, M.D. (2012). Hippocampal activity during recognition memory co-varies with the accuracy and confidence of source memory judgments. Hippocampus, 22, 1429-1437),
participants studied pictures presented to the left or right of central fixation. In a later test phase, participants underwent functional magnetic resonance imaging (fMRI) where studied and new pictures were presented in central vision. For each picture, participants first made a remember, know, or new response. For each picture judged remember or know, they then signaled the item’s study location using a 6-point confidence scale (ranging from 'definitely-left' to 'definitely-right'). Hippocampal activity was graded as a function of the accuracy and confidence of source judgments associated with a remember response. Hippocampal activity, however, did not differ between remember responses associated with the lowest level of source confidence and know responses. Here, we assessed whether activity in the hippocampus or other members of the ‘core recollection network’ dissociated according to whether test items elicited a phenomenal sense of recollection (a remember judgment) or an accurate source judgment. We reanalyzed the previous dataset to create four response categories which dissociated ‘phenomenal’ and ‘objective’ recollection: remember-accurate source, remember-inaccurate source, know-accurate source, and know-inaccurate source. A main effect across the parameter estimates associated with each of the response categories revealed differential activity within the hippocampus, angular gyrus, precuneus, and striatum. A follow-up ANOVA with factors of region and response category revealed a significant region by response category interaction. In the hippocampus, remember-accurate and know-accurate responses elicited greater activity than remember-inaccurate and know-inaccurate responses. In all other regions, the general pattern was of greater activity for remember-accurate and remember-inaccurate responses relative to know-accurate and know-inaccurate responses. These results indicate that whereas the hippocampus is solely sensitive to the amount of contextual information retrieved, other regions are more sensitive to whether a test item elicits a phenomenal sense of recollection.

Disclosures: P.P. Thakral: None. S.S. Yu: None. M.D. Rugg: None.

Poster

526. Human Memory Retrieval and Reactivation

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Topic: F.01. Human Cognition and Behavior

Support: NIH Grant R01-MH094480

Title: Latent variable modeling of temporal profiles of neural activity during the processing of continuous natural stimuli
Authors: *M. CHOW*¹, J. CHEN¹,², U. HASSON¹;²

Abstract: In everyday life, information arrives continuously from the environment, and a multitude of mnemonic processes contribute to comprehension moment by moment. However, the complex nature of continuous natural stimuli (e.g., visual or auditory narratives) makes modeling the underlying neural dynamics especially challenging. While much research in fMRI has made use of the general linear model, which requires explicit predictions of BOLD activity across time, in natural stimuli the exact form that neural activity will take across time is difficult to predict. We used latent variable models to characterize neural activity across time during continuous natural stimuli (auditory/visual narratives) by measuring relationships between subjects, within and across conditions. In these models, observed neural timecourses were expressed as a noisy representation of an underlying, group-specific timecourse. We applied this approach to two datasets in which episodic memory was manipulated. Latent variable models enabled us to explicitly describe cortical and hippocampal dynamics during audio/visual narratives where: 1) subjects needed to retrieve episodic details from one day earlier; 2) subjects needed to retrieve episodic details from one minute earlier. In both datasets we were able to differentiate the memory-demanding conditions from control conditions that were perfectly matched for stimulus input. Thus, our latent variable model approach provides a powerful tool for exploring temporal profiles of neural dynamics that support episodic memory and comprehension during real-life continuous stimuli.

Disclosures: M. Chow: None. J. Chen: None. U. Hasson: None.

Poster

526. Human Memory Retrieval and Reactivation

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Topic: F.01. Human Cognition and Behavior

Support: 1RO1NS089729 (NIH-NINDS)

NSF GRFP

Title: Distributed cortical representations of visual features in perception and memory

Authors: *S. E. FAVILA*¹, R. SAMIDE¹, B. A. KUHL¹;²
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Abstract: Neural activity patterns that reflect stimulus features during perception are reactivated when that stimulus is retrieved from memory. Reactivation of high-level visual category information (e.g., face vs. scene) has frequently been observed in inferior temporal cortex, but there is also evidence for reactivation of lower-level stimulus information in early visual cortex (Bosch et al., 2014). Recent evidence also indicates that reactivation of event-specific information occurs in lateral inferior parietal cortex (Kuhl and Chun, 2014). At present, however, there remains ambiguity regarding the nature of reactivated representations in lateral parietal cortex and how these representations relate to those in high- and low-level visual cortex. Here, we compared activity patterns in occipital, inferior temporal, and lateral parietal areas across perception and retrieval. We asked human participants to learn 32 unique word-image pairs, where images were exemplars from eight object categories (backpacks, cups, fish, flowers, fruits, hats, insects, shoes) and four color categories (blue, green, red, yellow). After learning the word-image pairs, subjects performed two different tasks while undergoing fMRI scanning: perception and cued retrieval. During perception runs, subjects viewed the images (without word cues) while performing an orthogonal target detection task. During retrieval runs, subjects were presented with word cues and recalled the corresponding images. Multivariate pattern analysis showed that, in visual regions, feature sensitivity during perception was reinstated at retrieval. In both tasks, neural activity patterns in early visual cortex discriminated object colors while activity patterns in inferior temporal cortex and lateral occipital cortex discriminated object categories. In lateral inferior parietal cortex, activity patterns during retrieval carried information about both color and object categories. Additionally, comparison of neural activity patterns during perception and retrieval revealed that lateral inferior parietal cortex exhibited exemplar-level reinstatement. These findings suggest a role for parietal cortex in combining event features reinstated in distinct visual cortical areas into an integrated memory.

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Authors: *I. SINGH*\(^1\), A. OLIVA\(^2\), M. HOWARD\(^3\);
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Abstract: It is well-known that memory degrades over time. In continuous recognition the recency effect manifests as a decrease in accuracy and an increase in response time (RT) with the lag of a repeated stimulus. We used visual recognition with highly-memorable pictures to mitigate changes in accuracy and enable a detailed examination of the effect of the passage of time on retrieval dynamics. The recency at which pictures were repeated ranged over more than two orders of magnitude from about 4 s up to about 500 s. Analysis of the RT distributions showed that the time at which memories became accessible changed with the recency of the probe item. These results suggest that visual memories were stored on a timeline that was sequentially scanned. Because the time to access the memory depended on the logarithm of the recency this suggests a timeline with Weber-Fechner spacing.


Poster

526. Human Memory Retrieval and Reactivation

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 526.10/X25

Topic: F.01. Human Cognition and Behavior

Title: Identifying the core recollection network based on single-trial measures of neural pattern reactivation

Authors: *E. K. LEIKER*, J. D. JOHNSON;
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Abstract: Episodic memory retrieval engages a network of brain regions including the hippocampus, posterior parietal cortex, medial prefrontal cortex, and retrosplenial/posterior cingulate cortex. To date, this “core” network has predominantly been identified either by contrasting the neural correlates of behavioral indices of recollection or through resting-state functional-connectivity (FC) analyses. One potential limitation of the behavioral approach is that the contrasts typically involve only a few memory conditions (e.g., remember vs. know, source correct vs. incorrect), leaving open the possibility that the identified regions largely reflect broad differences as opposed to graded changes in recollection. Here, we employed neural measures of reactivating episodic memories, in combination with a pattern-based FC approach (Coutanche & Thompson-Schill, 2013), to facilitate the identification of brain regions that are sensitive to
recollection in a graded manner. Subjects viewed a series of words in the context of different encoding tasks and then completed a source-memory test. Single-trial measures of reactivating the task-related information at the time of retrieval were determined with multivariate pattern analysis (MVPA) and then correlated with voxel-wise activity throughout the brain. On the basis of the pattern-reactivation measure alone, we identified the regions of the core recollection network that have consistently been identified with behavioral analyses. In addition, a number of regions not typically associated with recollection - including lateral occipital cortex and lateral and anterior prefrontal cortex - were also revealed. These findings demonstrate that retrieval-related reactivation is useful in identifying a more expansive core recollection network than previously established, and they suggest that the enhanced sensitivity and continuous (i.e. single-trial) nature of neural patterns may prove effective, more generally, in improving the characterization of functional brain networks.

Disclosures: E.K. Leiker: None. J.D. Johnson: None.

Poster

526. Human Memory Retrieval and Reactivation

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Topic: F.01. Human Cognition and Behavior

Title: Memory reactivation during sleep promotes better consolidation of event episodes linked by overlapping memories

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Abstract: Targeted memory reactivation has proven to be an effective approach to strengthen individual memory episodes with high specificity. However, life experiences are overlapped in content. In our study, participants encoded two consecutive sets of different memories sharing a common element, where the second set of memories was sound cued during encoding. In a follow up nap, half of the sounds were presented during Non-REM sleep to promote the reactivation of a selected set of encoded episodes. We found higher recollection for those
memories that overlapped with those whose sounds were presented during sleep. Furthermore, sleep-specific neurophysiological response modulations, such as slow waves and neural oscillations at the delta and theta range (2-8Hz), were associated to the presentation of sounds related to the memory content. These findings support the idea that sleep promotes the emergence of interrelated memory networks by reactivating multiple event episodes that are linked by overlapping elements.


Poster

526. Human Memory Retrieval and Reactivation

Location: Hall A

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Topic: F.01. Human Cognition and Behavior

Support: NIH Grant 1R03HD073417-01

Title: Quantitative analysis of sleep and learning; insights into cortical development and retention abilities in infants


Abstract: Sleep in the first year of life is characterized by rapid behavioral and physiological changes. Neonatal stages of active, quiet, and indeterminate sleep are replaced by two stages: rapid-eye movement (REM) and non-rapid eye movement (NREM) sleep (Ednick et al., 2009; Jenni et al., 2004). A growing body of literature suggests that maturation of sleep reflects underlying cortical development (Colrain & Baker, 2011; Kurth et al., 2010). We also know that sleep aids retention of new information in older children (Gomez et al., 2006; Hupbach et al., 2009; Friedrich et al., 2015). Here we investigate if sleep supports retention of newly learned information in 6.5-month-old infants. We further investigate if sleep maturation relates to infant’s learning abilities. Thirty-six 6.5 month old infants were exposed to an artificial, statistical, language (Thiessan & Saffran, 2003) made up of 4 bisyllabic words, repeated in random order with no pauses. Infants were randomly assigned to a sleep or wake condition. Twenty-one infants slept while monitored polysomnographically (electrode placements at F3, F4, C3, C4, O1, O2), while fifteen remained awake as yoked controls for the sleep infants. After
approximately an hour and a half, all infants’ retention of the language was tested across two testing blocks. Previous literature shows that listening longer to ungrammatical strings demonstrates more advanced learning (Hunt, 1971). There was a significant interaction of learning block and grammaticality (F(1,28)=6.733, p<.02), such that infants had significantly longer listening times to ungrammatical than grammatical in the first block (t(29)=-2.209, p<.05), than the second (t(29)=1.112, p< n.s.). This demonstrates that infants can acquire and retain the linguistic information for at least a short period of time. Infants did not show a significant difference based on sleep condition (F(1,28)=.21, n.s.). Quantitative analysis of the polysomnogram data from infants in the sleep group showed a relationship between language learning in Block 1 and NREM EEG activity in the theta range (4.5-8Hz) at all electrode sites (p’s<.01), but only at F3, F4, and C4 with SWA (p’s<.05). Existing literature highlights a posterior to anterior shift of slow-wave activity (SWA) (Jenni et al., 2004; Kurth et al., 2010). While all infants show greater posterior SWA activity, those with more anterior activity show greater learning ability. Our results are the first to demonstrate a relationship between cortical maturation and learning ability, using sleep as a proxy for neural maturation.


Poster

526. Human Memory Retrieval and Reactivation

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 526.13/X28

Topic: F.01. Human Cognition and Behavior

Title: The effect of inhibitory brain stimulation on recognition-induced forgetting

Authors: *A. M. MAXCEY*1, J. BOSTIC2, H. GLENN3, C. LEGGETT3, R. M. G. REINHART4;
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Abstract: What are the consequences of accessing a visual long-term memory representation? Previous work has shown that accessing a long-term memory via retrieval improves memory for the targeted item and hurts memory for related items (Anderson et al., 1994). Recently we (Maxcey & Woodman, 2014) found a similar forgetting phenomenon with recognition of visual objects, a surprising finding because the typical assumption is that access to a memory representation during recognition is direct and uncomplicated. The popular inhibition account of
retrieval-induced forgetting posits that inhibition is required to overcome inhibition of competing items during practice, accounting for forgetting of related items (Anderson, 2003). Current theories of recognition memory assume that access occurs in recognition tasks without competition from related memory representations. If this is the case, what is the source of forgetting in our recognition-induced forgetting paradigm? In the present study we ask whether inhibition, the mechanism widely believed to be responsible for retrieval-induced forgetting, plays a role in recognition-induced forgetting. To examine the role of inhibition in our paradigm, we applied cathodal (hyperpolarizing) stimulation to the right dorso-lateral prefrontal cortex (site F4), believed to be engaged in inhibitory processing (Penolazzi et al., 2014). If inhibitory mechanisms are driving this forgetting effect, down-regulating dorso-lateral prefrontal cortex, and thus suppressing inhibitory mechanisms, should eliminate the forgetting effect in our paradigm. We found that cathodal transcranial direct-current stimulation of right dorso-lateral prefrontal cortex eliminated recognition-induced forgetting. These findings indicate that inhibitory mechanisms contribute to forgetting in recognition.


Poster

526. Human Memory Retrieval and Reactivation

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Topic: F.01. Human Cognition and Behavior

Support: Sapienza University grant prot. C26A11RESK

Title: Increased response competition does not affect implicit memory in schizophrenic patients

Authors: *C. M. ROSSI-ARNAUD*, V. REZENDE SILVA MARQUES *, P. SPATARO *, A. SCIARRETTA *, V. CESTARI *;
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Abstract: Schizophrenia is associated with severe deficits in explicit memory and working memory (Aleman et al., 1999; Forbes et al., 2009). In contrast, the extent to which schizophrenia affects implicit memory is less well known. Implicit memory refers to the unintentional retrieval of information encoded during the study phase and is often investigated through repetition priming tasks. An important distinction in the field is that between implicit tasks based on
identification (non-competitive) or production (competitive) processes (Gabrieli et al., 1999). Neuroimaging studies suggest that identification tasks require search processes dependent on the right cerebellum, whereas production tasks require selection processes dependent on the left frontal lobe (Desmond et al., 1998). Previous evidence indicate that schizophrenic patients exhibit reduced priming in production tasks (such as word stem completion and category exemplar generation), but not in identification tasks (like lexical decision and word-fragment completion) (see Marques et al., 2014). However, no study to date has directly tested the effects of response competition in the same implicit tasks in schizophrenic patients and healthy controls. The present study examines this issue in two experiments, with 46 schizophrenic patients and 58 controls. Experiment 1 assessed repetition priming in two versions of the word-stem completion task having few solutions (the low response competition condition) or many solutions (the high response competition condition). Similarly, Experiment 2 employed two versions of the verb generation task having one dominant verb response (the low response competition condition) or no dominant verb response (the high response competition condition). The results showed that repetition priming facilitated the performance of schizophrenic patients and healthy controls to the same extent in both tasks. Furthermore, schizophrenic patients were not disproportionally impaired by increases in response competition. These findings have relevant implications for understanding the functional integrity of the neural underpinnings of identification and production implicit processes in schizophrenia. This research was supported by Sapienza University grant prot. C26A11RESK

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Poster

526. Human Memory Retrieval and Reactivation

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Topic: F.01. Human Cognition and Behavior

Support: NIH RO1-MH087214

Title: Alpha oscillations track the content of representations retrieved from long term memory

Authors: *D. W. SUTTERER*, J. J. FOSTER*, J. T. SERENCES*, E. K. VOGEL*, E. AWH*;
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**Abstract:** Recent work has demonstrated that it is possible to reconstruct spatially-specific channel tuning functions (CTFs) during the encoding and delay period of a working memory (WM) task using a forward encoding model and electroencephalography. Specifically these CTFs can be derived from the distribution of alpha-band (8-12hz) activity across the scalp, providing a temporally resolved measure of the stored location. Here, we show that a similar decoding approach can be used to track the content and timecourse of representations retrieved from long term memory (LTM). Subjects learned randomly assigned positions for a collection of 120 unique shapes, with the position selected from a continuous 360 degree space around a circle. 24 hours after the initial learning session, subjects were presented with shape cues and asked to retrieve the associated position while EEG was recorded. We found robust spatially-selective CTFs could be obtained from the distribution of alpha-band (8-12hz) power ~600ms after the onset of the retrieval cue. These results suggest that holding representations retrieved from long term memory in mind relies upon a similar neural mechanism to that used to maintain information in spatial WM. Furthermore, these findings reveal a powerful approach for obtaining neural measures of LTM retrieval latency.


**Poster**

**526. Human Memory Retrieval and Reactivation**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

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**Topic:** F.01. Human Cognition and Behavior

**Support:** Guggenheim Fellowship

- Office of Naval Research Grant N00014-15-1-0033

**Title:** Oscillatory theta entrainment enhances source memory retrieval: Neural and behavioural consequences

**Authors:** *A. CLARKE, B. M. ROBERTS, J. CRIVELLI-DECKER, C. RANGANATH; Univ. of California Davis, Davis, CA

**Abstract:** Theta oscillations play a critical role in memory processes. During episodic retrieval, theta activity is increased for correctly remembered events and the context they were studied in (source memory) (Burgess & Gruzelier, 1997; Grubber et al., 2008). Moreover, recent evidence
shows that frontal theta power before a retrieval cue predicts subsequent source memory performance (Addante et al., 2011). This suggests that enhanced theta oscillations are not just a consequence of stimulus-evoked memory retrieval processes, but that the cognitive state prior to the retrieval cue also has an influence on memory performance (Rugg & Wilding, 2000). However, the current evidence linking theta oscillations and memory performance remains correlational. Here we sought obtain causal evidence that theta oscillations are critical for episodic memory retrieval, by manipulating the endogenous theta oscillations using an audio-visual entrainment paradigm. Scalp EEG was recorded while participants first encoded a series of words by making a pleasantness or animacy judgment. This was followed by 30 minutes of audio-visual entrainment where LEDs oscillated and tones pulsed at 5.5 Hz (theta group) or a control frequency (14 Hz). During the retrieval test, participants were presented with a mixture of previously encoded or new words and asked to indicate if the word was old or new (item memory) and in what context the item was previously studied. Resting EEG was recorded both before and after entrainment. We found that oscillatory entrainment influenced theta power in the post-entrainment resting EEG data for the theta group, with no changes for the control group. Critically, the theta group showed significantly better source memory performance than the control group. In the EEG, correct source memory judgements were found to have enhanced theta power over frontal electrodes which was greater for the theta entrainment group compared to controls. Our findings show that neural oscillations can be enhanced using sensory entrainment that has frequency specific effects. Entrainment causally altered theta activity, resulting in increased episodic memory performance, and demonstrates the importance of oscillatory theta activity for human memory processes.


Poster

526. Human Memory Retrieval and Reactivation

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Topic: F.01. Human Cognition and Behavior

Support: DFG: BU 2670/2-1

Title: Theta-alpha oscillations bind the hippocampus, prefrontal cortex and striatum during recollection: Evidence from simultaneous EEG-fMRI
Abstract: The recognition of previously encountered stimuli rests upon an assessment of stimulus familiarity. Some items, however, additionally trigger the recollection of qualitative information associated with the context of encoding. This process of recollective retrieval has primarily been linked with theta (4-8 Hz) power changes in electrophysiological activity (M/EEG) as well as BOLD effects in a network including the hippocampus and frontal cortex, raising the question how these phenomena are related. Although the notion of the hippocampus coordinating neocortical activity by synchronization in the theta range is common among theoretical models of recollection, direct evidence supporting this hypothesis is scarce. By combining EEG and fMRI during a remember/know recognition task, we can show in healthy human subjects that recollection-specific theta-alpha (4-13 Hz) effects at the scalp level are correlated with increases in functional connectivity (PPI) between the hippocampus and prefrontal cortex for recollected compared to familiar items. Interestingly, we observed the same relationship between theta-alpha power and hippocampal connectivity in the striatum, an area that has repeatedly been linked to retrieval success. Taken together, our results provide compelling evidence that theta-alpha oscillations functionally bind the hippocampus, prefrontal cortex and striatum during memory recollection.

**Authors:** *A. SHPEKTOR*¹, E. F. PAVONE², N. VUKOVIC¹, A. LEBEDEVA¹, M. FEURRA¹;
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**Abstract:** A polymorphism in the BDNF (BDNF val66met; rs6265) gene causing a valine (val)-to-methionine (met) substitution at codon 66 results in altered intracellular trafficking and packaging of BDNF, and in a reduction of its regulated secretion. BDNF-val/val carriers usually showed a better cognitive performance in terms of motor, attention and memory tasks with respect to those who are met-carriers. Not so far it has been shown that BDNFval66met genotype predicts variation in hippocampal anatomy as well as in human episodic memory function. So far it is still unclear if Val/Val subjects are susceptible to better plasticity and if their cognitive performance is a matter of better connectivity. Here we investigated how the BDNF val66met polymorphism affects cognitive memory performance in healthy young individuals. To do this we used a deep and shallow episodic memory task which is a classical paradigm to test the level of processing effect (LoP). Since val/val might be more susceptible to more stable plasticity change respect to Val/Met carriers, we used Repetitive Transcranial Magnetic Stimulation (rTMS) as interference approach to causally address the role of the left and right DLPFC during episodic memory retrieval with respect to the BDNF val66met polymorphism. To do this, we recruited two groups of healthy subjects (Val/Val and Val/Met carriers). Left and right DLPFC as well as a control site (Vertex) was targeted by time-locked rTMS at stimulus presentation during retrieval. Our findings indicated the causal involvement of the DLPFC in episodic memory by showing for the first time how rTMS at retrieval modulate the behavioral performance accordingly to the BDNF val66met.

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**Poster**

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**Topic:** F.01. Human Cognition and Behavior

**Support:** ERC-StG 261177

NWO-Vidi 452-12-009
Title: Directional coding during imagination

Authors: *J. BELLMUND, L. DEUKER, T. NAVARRO SCHROEDER, C. F. DOELLER; Donders Institute, Radboud Univ., Nijmegen, Netherlands

Abstract: Successful navigation requires information about both the organism’s current and target location as well as about the directional relationship between them. Electrophysiological recordings in rodents suggest that positional information is provided by place and grid cells, while head direction cells code for the animal’s allocentric facing direction. Recent neuroimaging studies in humans provide evidence for directional representations in the human brain on a coarse scale by discriminating the four cardinal directions. Here, we use fMRI and representational similarity analysis to examine more fine-grained representations of directions in a novel, virtual-reality direction-imagination task. Participants imagined directions between buildings in a realistic large-scale virtual-reality city. A parametric measure of directional memory was closely related to navigation performance during training and a post-scan map test. Pattern similarity analysis of fMRI data revealed fine-grained directional representations in medial temporal lobe regions associated with navigation. These findings shed light on how the human brain represents directional information, which is crucial for understanding navigational processes.


Poster

526. Human Memory Retrieval and Reactivation

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 526.20/X35

Topic: F.01. Human Cognition and Behavior

Support: ERC-StG 261177

NWO-Vidi 452-12-009

Title: Representational clustering of associative memories

Authors: *A. R. BACKUS¹, L. S. SCHURMANN¹, L. HILMER¹,², C. F. DOELLER¹;
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Abstract: During everyday life, we continuously acquire new knowledge through associative learning, shaping our network of interconnected memories. However, interrogating such mnemonic network representations in the brain using noninvasive neuroimaging techniques is challenging. Recent functional magnetic resonance imaging studies on implicit temporal regularity and inference learning have shown group-level shifts in mnemonic networks, by comparing neural activity patterns before and after learning. Here, we present a litmus test for this method, using a basic explicit paired-associate learning paradigm with fractal-like visual stimuli. We observe increased pattern similarity between associated stimuli compared to non-associated stimuli as a consequence of learning in the medial temporal lobe and medial prefrontal cortex. Moreover, we reconstruct the mnemonic networks of individual subjects to visualize representational clustering of paired associates. By demonstrating the ability to probe mnemonic networks and learning-mediated reconfigurations in the brain, we validate an important tool for memory research. In addition, this approach can potentially be leveraged to track learning in educational settings.


Poster

526. Human Memory Retrieval and Reactivation

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Topic: F.01. Human Cognition and Behavior

Support: ERC-StG 261177

NWO-Vidi 452-12-009

Title: Narrative remapping in the hippocampus

Authors: B. MILIVOJEVIC, M. VARADINOV, A. VICENTE GRABOVETSKY, *C. F. DOELLER;
Donders Institute, Radboud Univ., Nijmegen, Netherlands

Abstract: Essential components of episodic memories are the spatial and temporal context in which events took place. In our daily lives, we often revisit the same environments, and temporally proximal events are not necessarily related. Therefore, a more general context is necessary to organise our memories into networks of related events. Personal narratives may
provide such a general context unrestricted by space and time. But how does the brain enable the formation of such narrative-based contextual representations? One possibility is that hippocampal mechanisms which enable remapping of spatial contexts and their gradual divergence over time also support the formation of narrative contexts. To test this hypothesis, we let our participants watch Sliding Doors, a movie which consists of two interleaved narratives, while monitoring their brain activity using fMRI. We then leveraged representational similarity analysis, using correlations between across-voxel activity patterns as a proxy of neural similarity, to track the emergence of narrative-specific hippocampal representations over the course of the movie. The movie was segmented into TR-length time bins, and each segment was tagged for the following information: narratives, characters and locations. Results of a post-scanning narrative-discrimination task indicated that participants formed reliable narrative representations in memory. In our imaging analysis, we first determined if we can differentiate between the narratives in the hippocampus and that these narrative-context representations diverged gradually over time, with stable representations emerging only about an hour into the movie. Next, we identified the brain regions which can distinguish between exemplars of specific visual categories: people and locations. Unsurprisingly, regions in the ventral visual stream were sensitive to the within-category exemplar identity. Surprisingly, however, similar differentiation was also observed between exemplars in the hippocampus. These results suggest that the hippocampus not only differentiates between contextual narrative representations, but also between representations of separate items within narratives. These item-specific representations may reflect abstracted nodal representations, which emerge as a consequence of repeated exposure to characters and locations through multiple events. In conclusion, we provide first evidence for the gradual emergence of narrative context representations in the human hippocampus, akin to remapping-induced spatial context representations of rodent place cells.

Disclosures: B. Milivojevic: None. M. Varadinov: None. A. Vicente Grabovetsky: None. C.F. Doeller: None.

Poster

526. Human Memory Retrieval and Reactivation

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Topic: F.01. Human Cognition and Behavior

Support: ERC Starting Investigator Grant AGESPACE 335090
Title: Age-related recognition memory deficits explained by hippocampal pattern completion at 7T-fMRI

Authors: *P. VIEWEG†, C. BILSING†, J. FABER‡, R. STIRNBERG‡, D. BRENNER‡, T. STÖCKER‡, T. WOLBERS†,‡,§;
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Abstract: Accurate memory retrieval from partial or degraded input - i.e. when trying to find our way across a park with all the trees having lost their leaves - requires the reactivation of memory traces, a hippocampal mechanism termed pattern completion. Given its extensive excitatory recurrent connections, region CA3 within the hippocampus has been identified as a likely candidate to execute the auto-associative processing essential for pattern completion (Marr, 1971). However, age-related changes in hippocampal integrity have been hypothesized to shift the balance of memory processes in favour of the retrieval of already stored information (pattern completion), to the detriment of encoding new events (pattern separation; Wilson et al., 2006). In this study, we investigated the underlying neural basis of age-related pattern completion differences with 7T-fMRI, using a scene recognition paradigm we have recently established as a behavioral marker for hippocampal pattern completion (Vieweg et al., 2015). Healthy younger and older adults were asked to identify complete or partially masked scenes in the scanner, half of which they had learned previously. For both groups, recognition accuracy was reduced with decreasing stimulus completeness. This effect, however, was more pronounced in older adults, suggesting that pattern completion is adversely affected by aging. Intriguingly, despite this substantial age-related performance decline, older adults also showed a bias toward pattern completion when only partial information was available. Moreover, following the segmentation of hippocampal subfields on T2-weighted images, we performed multivariate pattern analysis to quantify the representational similarity for different levels of stimulus completeness and stimulus type (learned or novel). The results showed that the hippocampal subfields were differentially susceptible to our completeness manipulation, and their involvement differed between age groups. Taken together, our findings provide important insights into the neural representations that underlie pattern completion in the hippocampus elicited by partial information. Moreover, they can pave the way for developing a mechanistic understanding of memory impairments frequently observed in old age and in neurodegenerative diseases that affect hippocampal integrity.


Poster

526. Human Memory Retrieval and Reactivation
Title: Consolidating and expanding mnemonic networks

Authors: *S. H. COLLIN, B. MILIVOJEVIC, C. F. DOELLER; Donders Institute, Radboud Univ., Nijmegen, Netherlands

Abstract: One of the key features of episodic-memory formation is the integration of multiple events and experiences into coherent mnemonic representations. Over time, these mnemonic networks are often extended with new information. To simulate episodic-memory formation, we have previously used The Sims 3 to make videos of life-like animated events that could either be integrated into narratives or not. We showed increased neural similarity in the hippocampus and medial prefrontal cortex (mPFC) as a result of linking events into a narrative, which suggests the formation of a shared representation coding for linked events [1]. However, it remains unclear whether these ensuing mnemonic networks are also consolidated as unified narrative representations c.f. Ref. [2], and what are the neural mechanisms for expanding such narrative representations after consolidation. To study these outstanding questions, we re-invited the participants from Ref. [1] for a second fMRI session on the subsequent day. On this day, they were presented with the same events as before, with the addition of a new event which was introduced and extended the initial narratives. Results of representational similarity analysis, a multivariate analysis method that uses across-voxel activation patterns as a proxy of neural similarity, showed that Day 1 narrative representations in mPFC were still present on Day 2. However, narrative representations in posterior MTL disappeared on Day 2. Interestingly, a new integrated representation emerged in anterior MTL on Day 2. To examine the neural mechanisms underlying integration of novel information into the consolidated narrative representations, we correlated the across-voxel activation pattern of the novel events with the initial narrative events, showing increased neural similarity in mPFC. These findings suggest that episodic memories are consolidated as unified representations and can be extended with newly available information in mPFC, even after consolidation. This is in accordance with rapid schema formation known to occur in this brain region [3, 4]. However, anterior MTL seems to serve a complementary role in consolidating this expanded narrative, potentially by maintaining a large-scale narrative representation (Collin et al., SfN 2014, 2014-S-3754-SfN) until it is sufficiently strengthened in mPFC. References: [1] Milivojevic et al., (2015) Current Biology, 25, 1-10. [2] Kumaran &
Disclosures: S.H. Collin: None. B. Milivojevic: None. C.F. Doeller: None.

Poster

526. Human Memory Retrieval and Reactivation

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Topic: F.01. Human Cognition and Behavior

Support: ERC-StG 261177

NWO-Vidi 452-12-009

Title: Time is memory: dissociating temporal and sequence coding

Authors: *L. DEUKER, N. MONTIJN, L. SCHURMANN, C. F. DOELLER;
Donders Institute, Radboud Univ., Nijmegen, Netherlands

Abstract: Episodic memory, which is critically dependent on the hippocampus, has been defined as remembering what happened where and when. While there is a large body of evidence for the role of hippocampus in spatial memory (remembering what happened where), less research has been conducted to elucidate the role of hippocampus in temporal memory (what happened when). Recently, cells have been discovered in the rodent hippocampus, which specifically encode the temporal aspect of an episode. In humans, neuroimaging studies have shown that stimuli, which were temporally linked or that were shown close to each other in a predictable order exhibit higher neural pattern similarity. However, these studies did not investigate temporal memory per se, but used sequential order or presence within the same event boundaries as a proxy for temporal relatedness. In this study, we investigate the role of the hippocampus in representing temporal distance between events over and above the impact of the mere order in which events are shown. To this end, we developed a novel task using complex visual scenes depicting every-day events in the life of a virtual family, created with the life-simulation game ‘The Sims 3’. Events were presented as happening during four different virtual days, each event at a specific time. Events were spaced differently in these days, with some events only one virtual hour apart, while other events were separated by four hours. This allows us to dissociate order memory from temporal memory. Crucially, we scanned participants with functional magnetic resonance imaging while they were viewing the events in random order, both before
(“pre-phase”) and after (“post-phase”) they learned which day and time the scenes belonged to. We then compared the change in pattern similarity with representational similarity analysis from the pre-phase to the post-phase. Behaviorally, participants were well able to learn the timing of the scenes. Preliminary fMRI results show that pattern similarity in the hippocampus covaries with the temporal distance between events. These findings may shed new light on the role of the human hippocampus in remembering temporal event structures.

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**Poster**

526. Human Memory Retrieval and Reactivation

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**Topic:** F.01. Human Cognition and Behavior

**Support:** ERC-StG 261177

NWO-Vidi 452-12-009

**Title:** Navigating memories: how episodes and space combine in the hippocampal formation

**Authors:** *N. DE HAAS, L. DEUKER, B. MILIVOJEVIC, C. F. DOELLER; Donders Institute, Radboud Univ., Nijmegen, Netherlands

**Abstract:** Two core memory systems are spatial and episodic memory. Both memory systems rely on the same brain structure: the hippocampal formation. So far, it remains unclear what the exact relationship between spatial and episodic memory is and how the hippocampal formation supports these seemingly different functions. We test two prevalent but opposing hypotheses that either the hippocampal formation supports these two systems via a common coding mechanism or via parallel processing. To this end, we combined high-resolution fMRI with behavioural tasks leveraging virtual reality and a life-simulation game. In the current experiment, participants had to learn associations between objects and two spatial contexts (spatial task) as well as two episodic contexts (episodic task), respectively. The distribution of objects over spatial and episodic contexts resulted in four different types of object pairings in a 2x2 factorial design: pairs which share 1) a spatial context only, 2) an episodic context only, 3) both a spatial and episodic context and 4) no context. Before and after each task, we presented all objects in randomized order and recorded brain data. This allows us to detect changes of across-voxel pattern similarity for the different types of object pairs as a function of preceding task manipulation. We are then
able to test different predictions of the opposing theories concerning when and where changes in similarity occur for each type of pair in the hippocampal formation. Preliminary results of multivariate pattern analysis (n= 14) show an increase in neural similarity in the hippocampus for object pairs which either share a spatial context or an episodic context. This suggests that our experimental manipulation is powerful enough to induce changes in neural similarity between object pairs in the hippocampal formation and therefore suited to test predictions of the opposing hypotheses.

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Poster
527. Human Cognition: Networks and Dynamics

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Topic: F.01. Human Cognition and Behavior

Support: EPSRC Doctoral Prize grant

MRC project grant

Title: Structural connections of the medial prefrontal cortex: Dividing motor, semantic and default mode networks

Authors: *R. JACKSON, C. J. BAJADA, M. A. LAMBON RALPH, L. L. CLOUTMAN; Neurosci. and Aphasia Res. Unit, Sch. of Psychological Sci., Univ. of Manchester, Manchester, United Kingdom

Abstract: The medial prefrontal cortex (mPFC) is implicated in multiple domains, including the default mode network (DMN) and semantic processing. However, whether sub-regions are variably related to different domains, is unknown. This is the first exploration of the structural connectivity across the entire mPFC as a way to inform function. Structural connectivity analyses were performed on diffusion-weighted MR images from 24 participants. Unconstrained probabilistic tractography was seeded from ROIs of Brodmann areas 6, 8, 9, 10 and 11 using the PICo algorithm. As a follow up, tractography was computed for each voxel in ventromedial PFC (vmPFC). This allowed tractographic parcellation of the vmPFC using the PARCEllation of Neural Images using Pic algorithm. Differential connectivity was identified. BA6 connected to primary motor cortex and the corticospinal tract. BA11 connected to anterior temporal lobe (via the uncinate) and primary visual and auditory regions (via IFOF). BA9, positioned between these
extremes, showed local connectivity (frontal cortex and insula). BA8 and 10 had similar but reduced connectivity to BA6 and 11, respectively. In moving from BA6 to 11, a gradient of connectivity was demonstrated from motor through local to high-order cognition areas. However, mPFC subregions were not differentially connected to networks associated with distinct higher order functions (e.g., semantics vs. DMN). Therefore, a secondary analysis was conducted to parcellate the vmPFC (BA10 and 11) based on structural connectivity without user-defined ROIs. vmPFC voxels formed 2 clusters, with differential connectivity from basal BA11 (orbitofrontal cortex) and dorsal BA11 and BA10. The orbitofrontal cortex connected to temporal and occipital regions related to semantic and sensory processing, whereas more dorsal areas connected to DMN regions. Distinct areas of the mPFC are connected to regions involved in motor, semantic and default mode networks. The novel tractographic parcellation technique allowed an emergent division of regions with distinct functional roles.

Fig 1. A. The connectivity of each Brodmann area. B and C. Tractographic parcellation of the vmPFC. B. The seed voxels for each cluster. C. The corresponding connectivity maps.

Poster

527. Human Cognition: Networks and Dynamics

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 527.02/X42

Topic: F.01. Human Cognition and Behavior

Support: NSERC PDF

Title: Multivariate structure-function relationships in human brain networks

Authors: *B. MISIC1, R. BETZEL2, M. DE REUS3, M. VAN DEN HEUVEL3, M. BERMAN4, O. SPORNS2;
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Abstract: The emergence of spontaneous fluctuations in brain networks is shaped by the underlying anatomical connectivity patterns. Although the edge-wise correspondence between structural and functional connections has been convincingly demonstrated in multiple reports, little is known about how coherent functional network patterns emerge from structural networks. In the present study we move beyond the correspondence between individual structural and functional edges, and investigate the association between structural networks and functional networks. Data were derived from the Human Connectome Project (HCP), with n = 215 participants. Structural and functional data were parceled into 66 regions using the FreeSurfer atlas, and then further subdivided into 114 approximately equally sized parcels. Structural connectivity was estimated by applying computational tractography to diffusion tensor imaging (DTI) data, while functional connectivity was estimated as a Pearson correlation coefficient between functional magnetic resonance imaging (fMRI) time series, recorded in the absence of any overt task. Structure-function relationships were assessed using multivariate partial least squares (PLS) analysis. A structure-function covariance matrix was constructed by computing the covariance between all possible pairs of structural and functional connections across participants. The matrix was subjected to singular value decomposition, yielding a set of latent variables – weighted combinations of structural and functional connections that optimally covary with each other. The statistical significance of latent variables was calculated using permutation tests, while the statistical reliability with which individual connections contribute to latent variables was estimated using bootstrap resampling. We find five statistically significant patterns
(p < 0.001), reflecting robust combinations of structural and functional subnetworks that are optimally associated with one another. Importantly, the patterns generally do not show a one-to-one correspondence between structural and functional edges, but are rather rich and heterogeneous, with many functional relationships arising from seemingly unrelated, upstream anatomical connections. We also find that structural connections between high-degree hubs are disproportionately represented, suggesting that these connections are particularly important in establishing coherent functional networks. Altogether, these results suggest that the emergence of spontaneous fluctuations in neural activity occurs due to a complex combination of interactions on the structural connectome.


Poster

527. Human Cognition: Networks and Dynamics

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 527.03/X43

Topic: F.01. Human Cognition and Behavior

Support: Center for Advanced Imaging, NorthShore University HealthSystem

Title: Hippocampal connectivity with the visual and motor systems reflects the cognitive requirements of the task

Authors: *D. D. BURMAN1, T. D. HOPKINS2;
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Abstract: By synchronizing cortical activity, the hippocampus can influence widespread regions of cortex, including regions not implicated in its known roles of memory, spatial perception, and sensorimotor integration. Whether the hippocampus influences sensory and motor cortical areas, independent of its known roles in memory and perception, is unknown. In a group of twelve subjects, cortical activation was identified in two finger-tapping tasks (repetitive, remembered sequence) and two visual tasks (checkerboard, simple fixation). To identify task-specific patterns of hippocampal connectivity, psychophysical interactions (PPI) were computed between task conditions for each hippocampal voxel, and the magnitude of correlated activity was mapped in activated areas (sensorimotor cortex for motor tasks, occipital cortex for visual tasks). Sensorimotor connectivity was observed for both motor tasks, with peak connectivity during repetitive movements coinciding with each finger's topographic representation. Although visual...
activation extended into the periphery during the checkerboard task. hippocampal connectivity during visual tasks was limited to central vision. For all tasks, peak hippocampal connectivity among individuals (> 80% of maximum) typically mirrored the region of peak activation (> 80% of maximum), and peak connectivity from the left and right hippocampus overlapped. Unexpected based upon its known properties, these results suggest a role for the hippocampus in the cognitive control of intentional processes.


Poster

527. Human Cognition: Networks and Dynamics

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 527.04/X44

Topic: F.01. Human Cognition and Behavior

Support: NIH Grant MH096801

Title: Multivariate pattern analysis of resting state activity reveals spontaneously organized brain state dynamics

Authors: *R. H. CHEN¹, P. SHAFTO²,³, M. W. COLE¹;
¹Ctr. for Mol. and Behavioral Neurosci., ²Psychology, Rutgers University, Newark, Newark, NJ; ³Psychology and Computer Sci., Univ. of Louisville, Louisville, KY

Abstract: Resting state functional connectivity MRI is increasingly used to characterize functional networks in the human brain. We recently found that the resting state network architecture is present across a wide variety of tasks (Cole et al., 2014), suggesting the general functional relevance of resting state networks. This illustrates the importance of better understanding the processes underlying the organization of resting state neural activity. Recent literature has focused on resting state dynamics - variability in the structure of resting state activity across time. However, current methods use arbitrary temporal window lengths to characterize network configurations, despite rapid and variable transitions in spontaneous activity. Furthermore, we hypothesized that spontaneous dynamics are not restricted to functional connectivity changes - that they likely extend to distributed multivariate activation pattern changes as well. We therefore applied multivariate pattern analysis (MVPA), a popular method for task neuroimaging, to characterize global brain state transitions during resting state with fMRI (functional MRI). Similar to a form of MVPA called representational similarity analysis (Kriegeskorte, 2008), we assessed the similarity of brain activity patterns through time.
Unlike previous approaches, however, we applied this across all pairwise time points. When observing contiguous time points this revealed rapid transitions across multiple distinct but stable brain states. When observing non-contiguous time points we found that many of these multivariate patterns repeated across time (and across subjects), suggesting some brain states are spontaneously “revisited”. In addition to increasing our understanding of resting state dynamics and brain state transitions, this approach may open possibilities for future research by bringing a powerful class of methods developed for task neuroimaging to investigate resting state neuroimaging data. References: Cole MW, Bassett DS, Power JD, Braver TS, Petersen SE (2014) Intrinsic and task-evoked network architectures of the human brain. Neuron 83:238-251 Available at: http://dx.doi.org/10.1016/j.neuron.2014.05.014. Kriegeskorte N (2008) Representational similarity analysis - connecting the branches of systems neuroscience. Front Syst Neurosci Available at: http://www.frontiersin.org/Journal/Abstract.aspx?s=1091&name=systems_neuroscience&ARTDOI=10.3389/neuro.06.004.2008.


Poster

527. Human Cognition: Networks and Dynamics

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Support: ARC Grant SR120300015

ARC Fellowship FL110100103

ARC CE140100007

Title: Segregated and integrated brain dynamics underlying higher cognitive reasoning in humans

Authors: *L. HEARNE*¹, L. COCCHI¹, J. B. MATTINGLEY¹,²;
¹Queensland Brain Inst., St Lucia, Australia; ²Sch. of Psychology, Univ. of Queensland, St Lucia, Australia

Abstract: Human intelligence is defined as the ability to understand complex ideas, adapt effectively to the environment, engage in complex reasoning and overcome obstacles by taking thought. Early neuropsychological and lesion-mapping studies implicated frontal brain regions as
key to the processes underlying intelligence. More recently, the application of network-sensitive analysis techniques to neuroimaging data has extended this view by highlighting the importance of whole-brain patterns of neural activity and connectivity. In the current study we collected 7T fMRI data with high temporal and spatial resolution in a cohort of adult participants who also underwent intelligence testing. Participants’ brain responses were measured both at rest and during a novel non-verbal reasoning task akin to Sudoku. We examined the neural underpinnings of intelligence-related task performance using a combination of approaches sensitive to segregated changes in neural activity and integration between discrete brain regions. We found that reasoning evoked both increased and decreased neural activity and connectivity between widespread brain regions comprising several endogenous neural networks. Additionally, our results suggest a link between endogenous functional connectivity related to intelligence and changes in brain dynamics that arise due to increased cognitive reasoning demands. Taken together the findings suggest that reasoning processes required for high-level intelligence reflect widespread changes in neural dynamics across multiple brain networks.

Disclosures: L. Hearne: None. L. Cocchi: None. J.B. Mattingley: None.

Poster

527. Human Cognition: Networks and Dynamics

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Support: MEXT/JSPS KAKENHI Grant 19002010, 24220008 to Y.M.

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JSPS Research Fellowships for Young Scientists 234682, 265926 to K.M.

MEXT/JSPS KAKENHI Grant 26890007 to R.S.

Title: Hub-centric prefrontal network predicts lesion-effective site for contextual memory in macaques

Authors: *T. OSADA*1,2, Y. ADACHI1, K. MIYAMOTO1, K. JIMURA3, R. SETSUIE1, T. WATANABE1, Y. MIYASHITA1;
Abstract: Neuroimaging and neurophysiological studies have revealed that multiple areas in the prefrontal cortex (PFC) are activated in a specific memory task, but severity of impairment after PFC lesions is largely different depending on which activated area is damaged. No approach has been established to localize the lesion-effective site among the activated areas. We hypothesized that task-activated PFC areas form an ordered network centered at a task-specific "functional hub", and the lesion-effective site corresponds to the "functional hub", but not to a task-invariant "anatomical hub" in a static anatomical network. Here, we conducted fMRI experiments in two macaque monkeys performing temporal-order judgment with a list of visual stimuli in a 4.7-T MRI scanner (Osada et al., 2015). After serial presentation of a stimulus list, the monkeys were simultaneously presented with two of the listed stimuli and were required to select which stimulus had appeared more recently. Behaviorally, significant differences in correct response rates and reaction times due to the inclusion of end stimuli were observed (p < 10^-4, paired t-test), indicating that the cognitive demands varied depending on whether the end stimuli were included or not. We compared cortical activity during high and low demand judgment, and identified significant activations, including multiple bilateral PFC areas (p < 0.05 FWE corrected). Based on task-evoked connectivity among the activated areas calculated by psychophysiological interaction, we found that the activated areas formed a hub-centric network. The task-specific functional hub assessed by betweenness centrality (area 9/46d) corresponded to the well-documented lesion-effective site, avoiding the neighboring non-lesion-effective site. We also evaluated the anatomical network among the activated areas with the aid of CoCoMac database, and found that the anatomical hub (area 8Ad) did not correspond to the lesion-effective site. Quantitatively, with a novel simulated-lesion method based on support vector machine, the predicted severity of impairment was proportional to the hubness of the lesioned area in the functional network, rather than in the anatomical network. These results support our functional hub hypothesis and suggest that PFC areas dynamically shape a hub-centric network to reallocate the lesion-effective site depending on the cognitive processes, apart from anatomical hubs. Reference: Osada T, Adachi Y, Miyamoto K, Jimura K, Setsuie R, Miyashita Y (2015) Dynamically allocated hub in task-evoked network predicts the vulnerable prefrontal locus for contextual memory retrieval in macaques. PLOS Biology, in press


Poster

527. Human Cognition: Networks and Dynamics

Location: Hall A
An EM algorithm to predict cognitive state dynamics from behavioral signals

**Title:** An EM algorithm to predict cognitive state dynamics from behavioral signals

**Authors:** *A. YOUSEFI*¹, A. C. PAULK¹, T. DECKERSBACH², N. NOSSENSON¹, D. I. VALLEJO³, S. S. CASH¹, D. D. DOUGHERTY³, E. N. ESKANDAR¹, A. S. WIDGE², S. S. CASH¹, D. D. DOUGHERTY², E. N. ESKANDAR¹, A. S. WIDGE², U. T. EDEN⁴;

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**Abstract:** A critical challenge in neuroscience relates to the identification of the essential cognitive processes that shape perception, learning, memory, and behavior. Such cognitive processes are often dynamic, evolving through time or over the course of an experiment, and are typically inaccessible to direct measurement. Often, experiments are designed to allow investigators to infer features of a cognitive state by measuring various behavioral responses to stimuli. For example, in a learning task, researchers might use reaction time and response accuracy to infer how well a subject has learned an association. State space methods have been used successfully to model dynamic features of the neural and behavioral signals for a variety of cognitive tasks. Typically, such methods either make strong assumptions about the data structure, for example that the data are normally distributed and the cognitive process evolves linearly, or else are narrowly designed for a particular experimental paradigm. However, many classes of experiments include similar behavioral responses that are known to have distributions that are not well modeled as normal and dynamics that are not linear. For example, reaction times for simple tasks are non-negative and typically have asymmetric distributions. Many behavioral signals are either discrete, e.g. a binary response or multinomial decision, or a mixture of continuous and discrete signals. In this research, we propose a general mathematical framework to estimate cognitive state processes using common behavioral signals that include a range of distributions. Under this framework, the cognitive process is modeled as a time-dependent state variable, and the conditional distributions of the behavioral signals are modeled as a function of the cognitive process and experimental factors. We propose an adaptive filtering solution to simultaneously infer the model parameters and estimate the cognitive state process through the course of an experiment. The adaptive filter consists of a signal-smoother and an EM algorithm, which sequentially estimates model free parameters and the cognitive state variable to maximize the likelihood of the observed behavioral signals. The algorithm has been applied in two behavioral datasets; using the algorithm, the moment-to-moment variation in the subject behavior and its underlying cognitive process are captured. We derived the prediction algorithm.
for observations with gamma, Bernoulli, and mixture of gamma and Bernoulli distributions; the proposed methodology can be extended to other behavioral signals with log-normal, Poisson, or multinomial distributions, or mixture of these distributions.


Poster

527. Human Cognition: Networks and Dynamics

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        Alfred P. Sloan Foundation (DB)

Title: Feature-based attention during sequential tasks

Authors: *T. M. DESROCHERS¹, A. G. COLLINS¹, D. BADRE¹,²;
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Abstract: We complete many sequential tasks in the course of every-day life that require the selective allocation of attention as one progresses through sub-goals towards a common end goal. For example, when making a sandwich, attention could be directed towards color as a feature when selecting a tomato or towards shape as a feature when slicing the bread. Previous studies have shown that cuing the to-be-attended feature modulates the activity in the corresponding extrastriate cortex. However, there is debate as to whether these effects are the result of top-down or bottom up processes, given that most of the previous work used experimental designs where the feature to be attended was predictably blocked. We designed an experiment to examine these attentional processes in the context of simple sequences of tasks. Human participants were asked to sequentially repeat four judgments (e.g. color, shape, shape, color) in a block. Importantly, the dimension to be attended to was provided only by the internal sequence
representation, and the specific feature (e.g. red or blue) was random for each stimulus. Additionally, we simultaneously manipulated the stimulus features available and provided participants “clues” as to the currently relevant task/dimension on approximately one third of the trials by presenting a feature that was not an option for selection in the irrelevant dimension (e.g. green on a “shape” trial). Preliminary results indicate that a large network of frontal, parietal, and extrastriate cortical areas are activated on trials which participants received a clue versus no clue, despite no overall effect of clue trials on reaction times. We will also discuss follow up analyses that seek to dissociate patterns of activation related to bottom-up attentional capture mechanisms versus putative top-down feature based selection.


Poster

527. Human Cognition: Networks and Dynamics

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 527.09/Y1

Topic: F.01. Human Cognition and Behavior

Title: Cortical patterns of alpha power in auditory sensory memory

Authors: *A. WILSCH¹, M. J. HENRY¹, B. HERRMANN¹, J. OBLESER¹,²; ¹Max Planck Inst. For Human Cognitive and Brain Sci., Leipzig, Germany; ²Dept. of Psychology, Univ. of Lübeck, Lübeck, Germany

Abstract: The neural and behavioral dynamics of decay in working memory and sensory memory are not well understood. Increased memory load, for instance, is accompanied by increased alpha power (8–13 Hz) during memory retention. However, it is not known how alpha power across brain areas is modulated during memory decay, and whether a priori factors such as temporal expectation for to-be-remembered auditory information can counteract this memory decay. We here present data from two samples, using magnetoencephalography (N=20) and behavioral data modeling (N=19) in order to assess memory decay in auditory sensory memory. First, in an auditory delayed matching-to-sample task, we manipulated temporal expectations (fixed vs. jittered cue–stimulus time interval) for a pair of pure-tone sequences (S1–S2: discriminability individually adjusted) embedded in white noise and varied the duration of the delay between S1 and S2 (1, 2, 4 s). Longer delays impaired memory performance (analyzed using signal detection theory and area under the ROC-curve; Aₜ), but this impairment was less strong for fixed than for jittered S1 onset-time. Longer delays also modulated alpha power (analyzed using Fast Fourier transform and an adaptive spatial beamformer for source analysis);
Alpha power declined parametrically with longer delays in visual cortex, inferior parietal lobe, and precuneus. However, alpha power decreased relatively less after temporally expected S1 onsets compared to unexpected S1 onsets in left supramarginal gyrus. Across conditions, individual modulation of alpha power predominantly emerging from these areas predicted individual performance modulation. Second, to study the impact of temporal expectations on memory decay more precisely, we behaviorally tested six different delay phases (0.6–7.0 s) in a second sample. An exponential decay function with independent growth and decay terms was fitted to the performance measure $A_z$, separately for fixed and jittered trials. Fixed-foreperiod trials were associated with a significantly larger estimated growth term indicating enhanced maintenance of the representation after temporally expected compared to unexpected S1 onsets. To summarize, temporal expectations counteract decay in auditory sensory memory. Specifically, temporal expectations improve memory performance, while attenuating the alpha power decline as memory decays. Moreover, cortical patterns of the alpha power effects revealed by source analyses encourage a more nuanced perspective of the inhibitory role of alpha power.

**Disclosures:** A. Wilsch: None. M.J. Henry: None. B. Herrmann: None. J. Obleser: None.

**Poster**

527. Human Cognition: Networks and Dynamics

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 527.10/Y2

**Topic:** F.01. Human Cognition and Behavior

**Support:** National Institute of Neurological Disorders and Stroke (R01NS078396)  
US National Science Foundation (BCS1358907)

**Title:** Probing functional connectivity between default and salience networks using single pulse electrical brain stimulation

**Authors:** *J.-C. HSIANG*¹², B. L. FOSTER², S. GATTAS², V. RANGARAJAN², J. PARVIZI²;  
¹Washington Univ. In St. Louis, Saint Louis, MO; ²Lab. of Behavioral and Cognitive Neurosci. (LBCN), Stanford Human Intracranial Cognitive Electrophysiology Program (SHICEP) Stanford Univ., Stanford, CA

**Abstract:** A direct functional relationship between the default mode network (DMN) and salience network (SN) has been proposed based on slow fluctuations of hemodynamic response.
However, given the limitation of this method, the direction of such connectivity remains poorly understood. Our current study utilized cortico-cortical evoked potentials (CCEP) to fill this gap of knowledge. In 4 human participants with focal epilepsy implanted with depth electrodes, we applied single pulse electrical stimulation to selected electrode pairs in DMN and SN regions. These sites were selected in each participating subject based on network identity information from rs-fMRI and electrocorticography data obtained from the same subject. The DMN sites included the retrosplenial cortex (RSC) and medial prefrontal cortex (MPFC) while the SN nodes contained the dorsal anterior cingulate cortex (dACC) and insula. Our findings suggest a robust connectivity within networks, but an intriguing pattern of connectivity between nodes of different networks. For instance, when the dACC was stimulated, evoked responses were consistently recorded in the RSC but not vice versa. Our findings provide another layer of information about the pattern of connections between different networks of the brain and raise questions and hypotheses that can be addressed with targeted studies in the future.

Disclosures: J. Hsiang: None. B.L. Foster: None. S. Gattas: None. V. Rangarajan: None. J. Parvizi: None.

Poster

527. Human Cognition: Networks and Dynamics

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

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Topic: F.01. Human Cognition and Behavior

Support: FAER MRTG-02/15/2010-Palanca

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William D. Owens Research Fellowship

Career Award at the Scientific Interface from the Burroughs-Wellcome Fund

Title: Spatiotemporal motifs of correlated interactions across resting state networks
**Authors:** M. KAFASHAN¹, S. CHING¹, *B. A. PALANCA²;
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**Abstract:** INTRODUCTION: Conventional correlation analysis of blood oxygen level dependent (BOLD) functional magnetic resonance imaging (fMRI) signals allow the brain to be segmented into multiple modules of functional-linked regions, referred to as resting state networks (RSNs). These RSNs mirror activation patterns evoked by behavioral tasks and represent statistically correlated neural activity spatially distributed over salient epochs of time. This approach obscures incompletely characterized temporal dynamics of interaction within and across RSNs during different arousal states. METHODS: Healthy human volunteers underwent BOLD fMRI during quiet wakefulness and while rendered unresponsive by 1.2 vol% sevoflurane. Stringent frame censoring of motion artifact and regression of whole brain global signal were applied to the BOLD signals. A winner-take-all algorithm was employed to select 1076 regions with > 90% probability of assignment to seven RSNs. Repeated pseudorandom resampling of these 6 x 6 x 6 mm regions was performed with 15 representing each RSN. BOLD time series were concatenated across subjects and both arousal states. Correlation trajectories for each region pair were calculated using a bounded Kalman filtering approach (11 second window). Using a K-means algorithm, we defined 8 orthogonal clusters with region pair membership defined by the strength of covarying correlation trajectories. RESULTS: Clusters of correlation trajectories (Figure 1) are maintained to varying degrees across arousal state over time. The clusters can be described as the following: (1) covarying high correlation trajectories of intra-RSN regions pairs (Cluster 1), (2) intermediate correlation trajectories within and between RSNs (Cluster 2 and 3), (3) weak correlation interactions between RSNs (Cluster 4 and 5), and (4) very weak correlation interactions between RSNs (Cluster 6, 7 and 8). CONCLUSIONS: Clustering based on covarying correlation trajectories yields structural motifs that may represent higher order patterns of interaction between RSNs.

**Disclosures:** M. Kafashan: None. S. Ching: None. B.A. Palanca: None.
Facilitating spatial working memory in patients with diabetic polyneuropathy by transcranial direct current stimulation

Abstract: Diabetes mellitus may lead to multiple complications, including diabetic polyneuropathy (DPN) and spatial working memory (SWM) deficits. Since DPN and SWM impairment can be two neurological entities, manifesting peripheral and central neuropathy respectively in diabetic patients, we investigated the SWM deficit of the DPN patients and intended to improve it by anodal transcranial direct current stimulation (tDCS) over right dorsolateral prefrontal cortex. This age- and education-matched intervention study enrolled patients with DPN (n=16) and control subjects (n=16). A forward- and backward-recall computerized Corsi Block Tapping task (CBT), both with and without a concurrent motor interference task was used to measure SWM capacity. Each participant underwent tDCS-CBT (2mA/35cm², 15 minutes) and sham-CBT (2mA/35cm², 30 seconds) sessions on two separate days in a pseudorandom counterbalanced order. The results showed that DPN patients had lower score of Montreal Cognitive Assessment (MOCA) and lower working memory span on Wechsler Adult Intelligence Scale-Fourth Edition (WAIS-IV) compared with control subjects, whereas there was no significant between-group difference on the general intelligence (WAIS-IV). The working memory span of backward recall CBT with concurrent motor interference was positively correlated with the nerve conduction velocity (NCV) value in DPN patients before tDCS treatment. However, this correlation diminished after tDCS treatment associated with the improvement in memory span of the low performers with worse NCV. The trends that DPN
patients presented lower memory span compared with the control subjects were consistent across all four conditions of CBT. Anodal tDCS improved the spans of backward recall and backward recall with interference among all subjects. Particularly, the spans of backward recall with interference were significantly improved after tDCS in DPN patients. This is the first study delineating the positive correlation between diabetic peripheral neuropathy severity and spatial working memory capacity, and the beneficial effect of tDCS on spatial working memory for DPN patients as well as the control subjects, that will be implicated and applicable to the clinical practice.


Poster

528. Working Memory Assessment and Modulation

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 528.02/Y5

Topic: F.01. Human Cognition and Behavior

Title: Integration of Ebbinghaus Functions, Signal Detection Theory, and the Weber-Fechner Law for understanding component processes of working memory

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Abstract: Objective: Working memory (WM) requires generation of a mental representation with a particular information load (i.e., amount) that is maintained over a temporal delay period, placing constraints on WM capacity. Component processes underlying WM performance as load and delay demands increase include i) the change in correct responses and errors as defined by Ebbinghaus functions; ii) the trade-off regarding information maintenance and segregation of signal and noise distributions characterized by signal detection theory; iii) the ease of distinction between a probe and encoded array, akin to a just noticeable difference (JND) as characterized by the Weber-Fechner Law; and iv) the efficiency of response selection as indexed by reaction time (RT). In 2 independent experiments (Exp1, Exp2) we used a modified Sternberg Recognition paradigm with variable load and delay demands and conducted iterative modeling to map relationships among component processes of WM. Methods: Two cohorts of healthy young
adults (Exp1 n=56, Exp2 n=50) completed a computerized visuospatial recognition task in which information load (Exp1 1,3,5, 7 items, Exp2 3,5,7 items) and delay length over which information was held (Exp1 2.25 - 5.75s, Exp2 1.50 - 9.50s) varied. After briefly viewing an encoding array and following a variable delay interval, subjects responded whether a probe item matched the spatial location of any item in the encoding array. Hits/misses (signal trials), false alarms (FAs)/correct rejections (noise trials), and response time (RT) were recorded. d’ and B were calculated (from hits and FAs) for signal detection measures as was a JND metric which reflected the spatial distance between a probe item and the nearest item in the encoding array. Separate statistical models evaluated i) the effects of load, delay, JND, d’ and B on RT, and ii) the effects of load, delay, JND, and response time on hits and FAs. Results: For signal trials, RT was predicted by load, JND, d’ and B but not delay, while RT on noise trials were predicted by load, JND, and d’ but not B. In turn, hits were predicted on signal trials by load, delay, JND, and RT, whereas FAs on noise trials were predicted by these same terms except for delay. Discussion: Findings from these experiments provide a novel approach for unpacking and integrating component processes of WM and suggest: i) load and JND predict RT, ii) load, delay, JND, and RT predict hits, iii) load, JND, RT predict FA, and iv) d’ and B generally predict RT. These observations argue there are quantifiable relationships (i.e., a function space with feedback) among these processes that underlie the variation in capacity of this core cognitive ability.

component that reflects the deployment of attention (Kumar, Soto, & Humphreys, 2009). In the present study, we first used behavioral measures to investigate whether attention is still guided towards WM-matching items after an instruction to forget them. Participants memorized a colored shape, which was followed by a cue that indicated whether it should be remembered or forgotten. Subsequently, participants searched for a tilted line among vertical distractor lines, each embedded within a colored shape. The interval between the cue and the visual search task (ISI) was 200, 600, 1000 or 1400 ms and on some trials, one of the distractors in the search task matched the earlier-memorized object. The results showed that the matching distractor captured attention regardless of whether it had to be remembered or forgotten, but the capture effect by to-be-forgotten distractors was smaller. In addition, the capture effects were independent on the interval separating the cue and the search array. Taken together, these results suggest that an instruction to forget an earlier-memorized object attenuates but does not fully abolish memory-driven capture. In a subsequent EEG study we modified experimental procedure in order to directly measure the maintenance of the to-be-remembered and to-be-forgotten distractors in WM. Previous studies provided evidence that contralateral-delay activity (CDA) is a component that indexes active maintenance of object representations in WM (Carlisle, Arita, Pardo, & Woodman, 2011). Therefore, the experimental procedure in the ERP study was identical to the behavioral experiment except that a word cue was presented at the beginning of a trial, indicating whether the colored shape to the left (LEFT) or to the right (RIGHT) of the fixation cross had to be memorized. The ISI between the cue and the visual search task was 200 or 1000 ms. Electrophysiological correlates of the WM-matching distractors that had to be remembered or forgotten will be explored with ERP analysis, focusing on N2pc component that is measured during the visual search task and CDA component that is measured after the presentation of the word cue.

Disclosures: E. Sasin: None. M. Nieuwenstein: None.

Poster

528. Working Memory Assessment and Modulation

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 528.04/Y7

Topic: F.01. Human Cognition and Behavior

Support: European Research Council (336152)

Title: Investigating the role of attention in the time course of visual working memory
Authors: *J. JACOB*, C. JACOBS¹, B. G. BREITMEYER², J. SILVANTO¹;
¹Psychology, Univ. of Westminster, London, United Kingdom; ²Psychology, Univ. of Houston, Houston, TX

Abstract: We compared scanning of working memory for simple geometric shapes in cued (location, neutral) and non-cued (control) conditions to investigate the role of attention in the time course of visual working memory. A memory array of 4 items preceded a probe item at varying inter-stimulus intervals (ISIs), ranging from 520 to 5000ms, in the control condition, and in the cued conditions the array preceded a cue at varying ISIs, followed by the probe after 300ms. The location cue pointed to one of the 4 array items, either matching or mismatching the probe. The neutral cue pointed toward all four locations of the memory array. Observers reported whether the probe matched or mismatched the memory array. Response reaction times were collected, and comparison effects (CE) were computed by subtracting average reactions times for matched from mismatched memory array-probe pairings. Similar to previous findings (Jacob, Breitmeyer & Treviño, 2013), CEs in the control condition varied systematically across ISIs, likely reflecting fluctuations in attention and working memory content. The CEs in the location-cued condition followed the same temporal pattern as the control condition, but with dampened fluctuations, terminated by 4000ms. As expected, the location cue utilizes spatial attention to compare the cued item and the probe, reducing memory load. This is likely to reflect spatial attention overriding the effects of attention to WM content. In contrast, the CEs in the neutral-cued condition showed CE fluctuations with higher amplitude for later ISIs. The neutral cue enhances processing of the content of working memory, amplifying CEs as ISIs increase. Our results suggest that attention plays a role in in determining stages of information processing in working memory.

Disclosures: J. Jacob: None. C. Jacobs: None. B.G. Breitmeyer: None. J. Silvanto: None.

Poster

528. Working Memory Assessment and Modulation

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 528.05/Y8

Topic: F.01. Human Cognition and Behavior

Support: NIH R01 DK082803

Title: Dietary saturated fat versus monounsaturated fat has reversible effects on brain function and the secretion of pro-inflammatory cytokines in young women
**Authors:** *J. A. DUMAS*, J. BUNN, J. NICKERSON, K. CRAIN, D. EBENSTEIN, E. TARLETON, J. MAKAREWICZ, M. E. POYNTER, C. L. KIEN;  

**Abstract:** Our previous studies indicated that lowering the typically high intake of palmitic acid (PA) in the Western-patterned diet was reversibly associated with increased physical activity and decreased inflammatory stress. Physical activity can be mediated by executive functioning, and inflammatory signaling may impair cognition. Therefore, we hypothesized that dietary PA and oleic acid (OA) might differentially alter activation of brain regions associated with regulation of executive functioning as well as innate immunity. Thus, in 12 female subjects participating in a randomized, cross-over trial comparing 3-week high PA (HPA) and low PA/high OA diets, we evaluated functional magnetic resonance imaging (fMRI) using an N-back test of working memory, cytokine secretion by lipopolysaccharide-stimulated peripheral blood mononuclear cells (PBMC), and plasma cytokine concentrations. During the HPA diet compared to the HOA diet, activation in the caudate and putamen ($p < 0.005$) was relatively increased, PBMC secretion of interleukin (IL)-18 was higher ($p = 0.015$) and IL-1β trended higher ($p = 0.066$), and plasma concentrations of both IL-1β and IL-6 were higher ($p < 0.05$). Thus, effects of the HPA diet included less efficient brain functioning, the production of pro-inflammatory cytokines *in vivo*, and enhanced secretion of inflammasome-regulated cytokines from LPS-stimulated leukocytes *ex vivo*. This novel human study shows an inter-relationship between dietary fatty acid-induced changes in brain activation and pro-inflammatory cytokine production from circulating leukocytes.


**Poster**  
528. Working Memory Assessment and Modulation  
**Location:** Hall A  
**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM  
**Program#/Poster#:** 528.06/Y9  
**Topic:** F.01. Human Cognition and Behavior  
**Support:** SFB936/A3/B2

ERC-2009-AdG-249425
Title: Load-dependent versus training-induced power changes within the working memory network - An EEG study

Authors: *H. GUDI¹, J. M. RIMMELE², P. BRUNS¹, A. K. ENGEL², B. ROEDER¹;
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Abstract: Since the brain comprises highly interconnected networks, neuroplasticity as a consequence of training must be investigated at a network level rather than by focusing on local changes. In the present study we investigated neuroplasticity as a following of an extensive working memory (WM) training. An adaptive n-back paradigm was applied in two groups of healthy participants trained with either voice stimuli (group 1) or tactile stimuli (group 2). A control group performed a 1-back task (group 3). The EEG was recorded prior to and after a four sessions lasting training while participants performed an auditory 2-back-task (i.e., with the voice stimuli used during the training sessions in group 1). In the post-training session, participants additionally performed an adaptive n-back task to assess neural network activity while performing at their limits. A pre-post sessions comparison of neural oscillations elicited in the 2-back task revealed an increase in theta-band power at fronto-parietal channels after training in both groups 1 and 2, as compared to the control group 3. Additionally, to address load effects, oscillatory activity was compared between the low load (2-back task at post-training session) and high load (individually adapted n-back task at post-training session) conditions. A main effect of load was observed across all groups (1, 2, 3) indicating a decreased posterior-temporal but increased frontal theta-band power in the high load condition as compared to the low-load condition. Our findings are in accord with previous work relating enhanced executive demands to increased frontal theta-band power and a higher familiarity with the stimulus material to a reduction in the posterior theta activity. Interestingly, the neural correlates of group-specific training effects differed from those of group-independent load effects suggesting partially different neural mechanisms. Conflict of interest and funding The authors declare no competing financial interests. This study was supported by the DFG (SFB936/A3/B2) and the EU (ERC-2009-AdG-249425; ERC-2010-AdG-269716).


Poster

528. Working Memory Assessment and Modulation

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM
Title: Response speed and contextual congruency affect visual short term memory introspection

Authors: *C. JACOBS, J. JACOB, J. SILVANTO;
Univ. of Westminster, London, United Kingdom

Abstract: Metacognition is a form of introspection in which one reflects upon one’s own cognitive processes. Multiple factors modulating metacognition in long-term memory (LTM) have been identified (Koriat, 2007), but less is known about what influences metacognition of working memory. Here, we investigated the effects of two factors known to modulate LTM metacognition and/or LTM task performance on metacognitive performance (i.e. the difference between introspective ratings for accurate - inaccurate trials) in a visual short-term memory (VSTM) task. In experiment 1, we investigated the effect of reaction times (RTs), because, in LTM, trials with shorter RTs tend to be endorsed with higher confidence ratings (Kelley & Lindsay, 2003). Participants were tested on their memory for the orientation of Gabor patches over a 5 second delay period. Response speed was manipulated by artificially speeding up participants’ RTs or by slowing them down. After their initial response, participants rated on a 4-point scale either vividness of their memory, or confidence in their response. In both the confidence and vividness groups, metacognitive sensitivity decreased for each experimental condition compared to baseline, possibly due to the additional working memory load of having to remember an additional task rule. In experiment 2, we investigated the effect of context, because we hypothesized that this would differentially affect the vividness and confidence groups. The task was identical to experiment 1, but this time cue and test stimulus were surrounded by coloured rings, either of the same (i.e. congruent context) or different (i.e. incongruent context) colour. Confidence ratings, but not vividness, showed increased metacognitive sensitivity when the context changed compared to when the context at encoding and retrieval remained identical, possibly due to the alerting effect of the contextual change. However, accuracies were unaffected. These results are in accordance with earlier studies that also indicate that introspection and metacognitive assessment of visual working memory are more than just a read-out of the original memory trace (Bona et al., 2013; Bona & Silvanto, 2014).

Disclosures: C. Jacobs: None. J. Jacob: None. J. Silvanto: None.
Title: Contralateral delay activity tracks fluctuations in working memory success

Authors: *K. C. ADAM, E. K. VOGEL; Psychology, Univ. of Chicago, Chicago, IL

Abstract: Neural measures of working memory (WM) storage such as the Contralateral Delay Activity (CDA) are powerful tools in working memory research. CDA amplitude is sensitive to WM load, reaches an asymptote at known behavioral limits (3 items) and predicts individual differences in capacity. An open question, however, is whether neural measures of load also track trial-by-trial fluctuations in performance. Change detection tasks require estimation of performance across an average of many trials, but newer whole-report tasks allow measurement of performance for individual trials. Here, we took advantage of the single-trial nature of a whole-report task to test the relationship between CDA amplitude and working memory performance. If WM failures are due to decision-based errors and retrieval failures, CDA amplitude should not differentiate good and poor trials within a single set-size. On the other hand, CDA amplitude should clearly track performance if WM failures are due to storage failures. Experiment 1 (N = 25) validated the whole-report working-memory measure with a bilateral CDA design. As expected, CDA amplitude tracked set-size, reaching an asymptote at 3 items. Additionally, load-dependent N2PC selection effects were observed. In Experiment 2 (N = 37), we tracked fluctuations in performance by using only a single challenging set-size (6 items). The amplitudes of the N2PC as well as the CDA were higher (more negative) for high-performance trials compared to low-performance trials, suggesting that fluctuations in performance were related to the successful selection and storage of items.

Disclosures: K.C. Adam: None. E.K. Vogel: None.

Poster

528. Working Memory Assessment and Modulation

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM
(Un)conscious working memory? - Disentangling the relationship between conscious perception and working memory

Authors: *D. TRÜBUTSCHEK¹,²,³, S. MARTI¹,⁴, A. OJEDA¹,⁴, S. DEHAENE¹,⁴,⁵; ¹Inserm-CEA Cognitive Neuroimaging Unit, CEA Saclay, Gif Sur Yvette, France; ²Univ. Pierre et Marie Curie, Paris, France; ³Ecole des Neurosciences de Paris, Paris, France; ⁴Univ. Paris-Sud, Gif Sur Yvette, France; ⁵Collège de France, Paris, France

Abstract: Working memory (WM) refers to the temporary maintenance and manipulation of internal representations. Historically, it has been regarded as inextricably linked to consciousness (Baars & Franklin, 2003; Baddeley, 2003; James, 1890), with subjective awareness and WM sharing similar neural networks and the ability to report their contents. Recent evidence questions this view: Consciousness may neither be compulsory for WM, nor may all of the contents of WM be reportable (Soto, Mäntylä, & Silvanto, 2011; Dutta et al., 2014; Soto & Silvanto, 2014). Instead, an “unconscious” WM (unWM) system might exist, which - outside the realms of subjective awareness - receives, maintains, and operates on information. We here sought to exploit behavioral and neural data to clarify the relationship between conscious perception and WM, test the existence of, and characterize the features of unWM operations. Specifically, we conducted an MEG/EEG study (N Exp1 = 23) in conjunction with two behavioral experiments (N Exp2 = 28, N Exp3 = 30) in healthy human subjects who performed a paradigm in which, after a delay (0 - 4.1s), the position (20 or 24 possible circular locations) and visibility (1 [unseen] - 4 [clearly seen]) of a masked target square had to be indicated. Preliminary analyses of MEG recordings revealed a de-synchronization in the alpha and beta bands over frontal sensors observed only in conscious but not in unconscious trials, showing that these types of trials relied on distinct brain mechanisms. Still, participants reported the correct target position better than chance, even when not having seen the target. This blindsight effect resisted the presentation of visible distractors (Experiment 1), suggesting that unconscious information may be maintained for several seconds and shielded against distraction. In addition, a simultaneous demand on conscious WM (Experiment 2) modulated (but did not extinguish) participants’ ability to indicate the correct target position. Unconscious WM may thus share the same resources as conscious WM. Crucially, blindsight persisted even when participants had to report a rotated target position (Experiment 3), indicating that, as expected of WM, unconscious information may be maintained and manipulated. These results support the notion that WM may not exclusively operate on conscious inputs and challenge current beliefs of the relationship.
between WM and consciousness. An unWM system may exist, allowing for the maintenance and manipulation of unconscious internal representations. Ongoing MEG analyses should reveal the neural mechanisms enabling the maintenance of sensory information under conscious and unconscious conditions.

Disclosures: D. Trübutschek: None. S. Marti: None. A. Ojeda: None. S. Dehaene: None.

Poster

528. Working Memory Assessment and Modulation

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 528.10/Y13

Topic: F.01. Human Cognition and Behavior

Title: Attenuation of the p300 in patients with psychosis using a novel serial order oddball paradigm

Authors: *J. AXELROD¹, W. C. HOCHBERGER¹, T. A. CARRATHERS¹, S. K. KEEDY², S. K. HILL¹;
¹Rosalind Franklin Univ. of Med. and Scien, North Chicago, IL; ²Clin. Neuropsychology and Psychopharmacological Res. Unit, Univ. of Chicago, Chicago, IL

Abstract: Against a background of generalized cognitive impairments, individuals on the psychosis spectrum also show a unique deficit in serial order recall. The P300 presents as a viable marker for the updating and maintenance of information in working memory. We previously validated this novel serial order recall ERP paradigm in healthy individuals showing differential attenuation in P300 amplitude across oddball and non-oddball trials. This is the first study to assess this P300 paradigm in psychosis patients. Findings indicate that this novel task successfully elicited a P300 response, albeit attenuated, in patients. Thus, this novel task can be used to evaluate the integrity of neural circuits underlying P300 generation and related working memory and attentional processes in psychotic disorders. Future studies can focus on the impact of cognitive demand with varying delayed and response output conditions on P300 amplitude in this novel task, as well as the relationship between serial order position and signal degradation in psychotic disorders.


Poster
Title: Cognitive interference effects during manual force production in adults with Type II Diabetes

Authors: *S. L. GORNIAK*, B.-C. LEE, J. WANG;  
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Abstract: The overall aim of this project was to evaluate the relationship between cognitive decline and outcomes in manual sensorimotor behavior in adults with Type II Diabetes (T2D). Recent studies show that adults with T2D experience significant declines in cognitive function compared to healthy controls. As cognitive function is a major component of many daily motor activities, there is a critical need to investigate the impact of T2D on cognitive-motor actions. Ten (10) community-dwelling T2D patients and ten (10) age- and sex-matched healthy controls were recruited and evaluated in this cross-sectional study (6 females and 4 males per group; age = 60 ± 7 years). Cognitive, sensory, and motor performance was evaluated in one testing session. Health state and metabolic data (eg. glycated hemoglobin values, A1c) were also collected. Mild cognitive impairment, particularly in the domain of working memory was found in the T2D group versus controls. Control participants did not show any evidence of cognitive deficits. A relationship between working memory performance and A1c was found. Decrements in both sensory and motor performance in the T2D group were also found, compared to controls. When T2D patients were asked to perform manual activities in conjunction with working memory tasks (dual-tasking), both manual and cognitive performance declined significantly versus control performance. These data are the first to suggest simultaneous cognitive-motor losses in community-dwelling patients living with T2D. Cognitive-motor losses likely play a significant role in T2D patients experiencing difficulty in performing basic activities of daily living, such as bathing, grooming, and feeding.

Disclosures: S.L. Gorniak: None. B. Lee: None. J. Wang: None.
Title: Revealing hidden states in working memory using EEG

Authors: *M. Wolff*, J. Ding, N. Myers, M. G. Stokes;
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Abstract: It is often assumed that information in working memory (WM) is maintained via persistent activity states. However, recent evidence indicates that information in WM could be maintained in an effectively ‘activity-silent’ neural state (Sreenivasan, Curtis, & D’Esposito, 2014). Silent WM is consistent with recent cognitive and neural models (Mongillo, Barak, & Tsodyks, 2008), but poses an important experimental problem: how can we study these silent states using conventional measures of brain activity? Here, we propose a novel approach that is analogous to echolocation: using a high-contrast visual stimulus, it may be possible to drive brain activity during WM maintenance and measure the WM-dependent impulse response. Specifically, we recorded electroencephalography (EEG) while participants performed a simple visual working memory task in which a single, randomly oriented grating was remembered. Crucially, a fixed, task-irrelevant ‘impulse’ stimulus was shown in the maintenance period in half of the trials. The electrophysiological response from occipital channels was used to decode the orientations of the presented gratings. While stimuli could be reliably decoded during and shortly after stimulus presentation, decoding accuracy dropped back close to baseline in the delay period. However, the visual evoked response from the task-irrelevant stimulus resulted in a clear re-emergence in decoding accuracy. Interestingly, although the impulse response pattern differentiated the WM-stimulus, the discriminative pattern did not match the patterns during memory encoding. Our experiment provides important proof-of-concept for a promising and simple approach to decode ‘activity-silent’ WM content using non-invasive EEG. Mongillo, G., Barak, O., & Tsodyks, M. (2008). Synaptic Theory of Working Memory. Science, 319(5869), 1543-1546. Sreenivasan, K. K., Curtis, C. E., & D’Esposito, M. (2014). Revisiting the role of persistent neural activity during working memory. Trends in Cognitive Sciences, 18(2), 82-89.

Disclosures: M. Wolff: None. J. Ding: None. N. Myers: None. M.G. Stokes: None.
Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 528.13/Y16

Topic: F.01. Human Cognition and Behavior

Title: Working memory during pregnancy: prefrontal and parietal eeg correlation

Authors: *M. L. ALMANZA, M. GUEVARA, M. HERNANDEZ-GONZALEZ; Inst. De Neurociencias, Guadalajara, Mexico

Abstract: Pregnancy is a dynamic process in which significant behavioral changes take place. A substantial proportion of pregnant women perceive that their memory is impaired during this period. Similar results have been obtained in laboratory studies and they suggest that working memory may be altered during pregnancy. So far, these studies have only used neuropsychological tests. We need to go deeper in the neural substrates of the underlying working memory in pregnant women; particularly the dorsolateral prefrontal cortex and posterior parietal cortex, as these brain structures are critical components within the neural circuitry underlying working memory. This is why the goal of the current work is to examine the relationship between working memory during the three trimesters of pregnancy and its association with characteristic changes in inter and intrahemispheric frontal-parietal EEG correlations. Forty women were divided into 4 groups: three included women in different trimesters of pregnancy (E1, E2 and E3) and a control group of nonpregnant women (CO). Prefrontal EEG derivations (F1, F2, F3, F4) and parietal (P3, P4) was recorded during a visuospatial (Corsi block-tapping task) and verbal (verbal digit span) working memory. It was found that the degree of coupling EEG prefronto-parietal during the working memory tasks is characteristic for each trimester of pregnancy. It suggests that while behaviorally in the three periods the participants presented an efficient execution for the tasks, they showed a different EEG correlation pattern. Especially in participants of groups E1 and E3, the performing of both tasks of working memory (verbal or visuospatial) was associated with a higher correlation of slow EEG bands in non-specialized hemisphere, while in the participants of groups E1 and E2 the prefronto-parietal EEG correlation of gamma band in the hemisphere specialized for each task (verbal-left hemisphere and visuospatial-right hemisphere) was presented. These changes in the degree of cortical coupling of slow and fast EEG bands could be associated with the inhibition of irrelevant tasks for the activity and the temporal organization of working memory and the maintenance and manipulation of information. These EEG correlation data give objective evidence of participation prefronto-parietal circuit and gamma band oscillation in modulating working memory in pregnant women.

Task related functional connectivity within varieties of complex skill learning

Two cognitively demanding fMRI tasks acted as a platform to examine game learning-related interactions in task-positive and task-negative brain networks. The hybrid block design tasks were a memory updating task (random N-back; Basak & Verhaeghen, 2011) and a task-switching paradigm with blocks for single and dual tasks (Basak et al., 2008). These paradigms allowed us to look at four task conditions - switching and updating information in working memory (WM), switching between two tasks (Dual Task), no switching requirements (Single Task) and Rest. We investigated functional connectivity using seed-to-voxel based analysis, for three specific brain networks: fronto-executive (FE), fronto-parietal (FP) and default mode network (DMN). Right Anterior Prefrontal Cortex, right Inferior Parietal Lobe and Posterior Cingulate Cortex were chosen as the seeds for the FE, FP and DMN networks, respectively (Voss et al., 2010, 2012). Thirty-one younger and 32 older adults played two types of computer games, one strategy and one shooter. Each game, irrespective of the type, needed about 5.5 min to play; thus yielding an average of 12 strategy and 12 shooter games in 1.5 hours. Game learning for each game was estimated by calculating composite z-scores of the game outputs, viz. levels reached, number of games played, final level won. Functional connectivity results for 63 participants, after controlling for age differences, indicated that the FE and FP networks differ substantially across the 4 conditions. The Dual and Single Task conditions, compared to the WM, resulted in a broader connectivity to regions of cognitive control, e.g., DLPFC and Supramarginal Gyrus. Importantly, individual differences in game learning predicted different connectivity for strategy vs. shooter. Strategy learning predicted broader connectivity in the FE and DMN in WM condition; shooter learning predicted broader connectivity in all 3 networks in both the Single and Dual conditions. Subsequently, separate regression analyses were conducted for each age group. For the younger adults, in WM condition, the FE connectivity (but not FP) was differentially predicted by strategy (Cingulate
Gyrus) vs. shooter (Superior Frontal Gyrus) learning. No significant relationships were observed in the older adults. In the Dual Task condition, older, not younger, adults showed broad connectivity in both the FE and FP networks predicted by both types of learning. These results suggest that the demands of cognitive control in a task may vary across the two age groups; this in turn may lead to differential connectivity for the two cognitive control networks (FE and FP) in relation to the two types of game learning.

Disclosures: M.A. O'Connell: None. C. Basak: None.

Poster

528. Working Memory Assessment and Modulation

Location: Hall A

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Program#/Poster#: 528.15/Y18

Topic: F.01. Human Cognition and Behavior

Support: NIH grant 5R37AG-006265-25

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Title: Motion-related noise in structural brain images may be revealed with independent estimates of in-scanner head motion

Authors: N. K. SAVALIA1, P. F. AGRES1, M. Y. CHAN1, K. M. KENNEDY1, D. C. PARK1,2, *G. S. WIG1,2;

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Abstract: Accurate measures of brain structure are essential to neuroimaging for anatomical localization, image registration, and the general study of functional brain organization across populations. However, recent evidence suggests that motion-contaminated T1-weighted magnetic resonance imaging (MRI) results in misestimates of brain structure (Reuter et al., 2015). Particularly alarming, many cross-cohort studies contrast individuals who differ in their degree of motion during MRI (e.g., children with ADHD vs. healthy controls, older vs. younger adults). Accordingly, this motion confound could systematically bias analyses of between group differences. Since conventional datasets collect T1 images without catchall prospective motion correction methods and limited direct measures of head motion, a practical alternative is needed to account for the potential motion-induced noise in measures of brain anatomy. We analyzed the head movements during functional MRI (fMRI) scanning of 266 healthy adults (20-89 years,
to identify stable features of in-scanner head motion. The sample comprised a subset of participants from the Dallas Lifespan Brain Study (DLBS) who completed the full DLBS neuroimaging protocol. The protocol allowed extensive quantification of head motion across a variety of scans with differing task demands while also acquiring a T1 structural image in the same session. We found magnitude of head motion increased with age and exhibited high within-subject stability across scan types. Comparing motion estimates to quality control (QC) ratings of T1 structural images revealed a strong relationship between the two, indicating one might be used to inform the other. Both QC ratings and measures of head motion predicted independent variance in general estimates of brain structure (e.g., FreeSurfer-derived cortical and volume-based morphometrics; Fischl, 2012), independent of the effects of age. Images believed to be susceptible to motion-related noise showed reduced structural estimates versus age- and gender-matched samples, leading to inflated effect sizes in the relationships between multiple anatomical measures and age. Of additional relevance, examining motion-contaminated anatomical scans revealed that the regions most prone to potential movement-related effects included many reported to undergo prominent atrophy with age. The findings suggest that problematic motion-related noise in structural brain scans may be retrospectively flagged and removed, in part, via a combination of techniques relying on rater-defined QC and estimates of motion obtained from independent scans collected during the same session.

Abstract: Objective: It is essential to develop a behavior scale easy to implement comparative and practical usage for neurosciences. This study evaluated the validity and reliability of the short form of the Interaction Rating Scale Advanced (IRSA-Brief) as a practical index of social competence development. Methods: Fifty adults completed a five-minute interaction session and were assessed with the IRSA-Brief and IRSA. Health social professionals evaluated their social competence based on regular practical assessments. IRSA-Brief is a shortened form of the IRSA. In this version, the six IRSA sub-scales are summarized by the three most common subscales of social competence: “coordination,” “self-regulation,” and “assertion.” The IRSA-Brief was developed by examining each of the original scale's 92 items, and choosing the most feasible items to represent each IRSA-Brief subscale in terms of content validity and psychometric acceptability. Two different sets of variables were scored for each sub-scale: behavior items and impression items. Each subscale assessed the presence of behavior (0 = no, 1 = yes) and the sum of all items in the subscale provided the overall behavior score. The total score range was from 0 to 39. Scores for the impression items and the overall impression item were on a five-point scale: 1 = not evident at all, 2 = not clearly evident, 3 = neutral, 4 = evident, 5 = evident at high level. The evaluator completed the 39-item checklist focusing on each behavior (e.g., expresses his/her own feeling to the partner). Inter-observer reliability was found to be 90%. The IRSA-Brief’s objective is to evaluate interactions in a short period of time in daily situations. Results: The results indicated that the IRSA-Brief scores had a moderately high correlation with the IRSA scores (r = 0.41) and the professionals’ practical evaluations (r = 0.72). Cronbach’s alpha was 0.84. Discussion: As the IRSA-Brief can measure social competence with high validity and reliability, providing evidence of social competence development. Conclusion: IRSA-Brief would be useful in practical settings to further assist with social competence development, support, and treatment in neurosciences.


Poster

529. Social Cognition: Behavior, Neural Basis and Pharmacology

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 529.02/Y20

Topic: F.01. Human Cognition and Behavior

Support: MOST 103-2410-H-010-003-MY3
                  104-2420-H-010-001-
                  RD2014-003
Title: Testosterone administration modulates neural responses to morally-laden scenarios in females

Authors: *C. CHEN;
Natl. Yang-Ming Univ., Taipei, Taiwan

Abstract: Several neuromodulators, including testosterone, influence moral cognition. However, the neural mechanism by which testosterone modulates moral reasoning remains to be determined. Using a placebo-controlled within-subject design, the current study examined the neuromodulatory effect of testosterone administration in young females by combining moral dilemmas, second-to-fourth (2D: 4D) digit ratio (a proxy of fetal testosterone), functional magnetic resonance imaging (fMRI), and subjective ratings on morally-laden scenarios. Results showed that acute testosterone administrations elicited more utilitarian response to evitable dilemmas, in which 2D: 4D predicted 22% of the variance. The activity in the amygdala, anterior insula, ventromedial, and dorsolateral prefrontal cortex (DLPFC) was increased to perceived agency in spite of comparable subjective ratings. The effective connectivity between amygdala and DLPFC was reduced. The activity in the temporoparietal junction predicted utilitarian responses to evitable dilemmas. The findings demonstrate acute effect of testosterone on neural responses associated with moral judgments. Administering a single dose of testosterone can modulate the capacity of emotional regulation and perspective taking, which, in turn, heightens reactivity to social provocation and to make utilitarian judgments. Keywords: moral evaluation; testosterone; utilitarian judgment; fMRI; agency

Disclosures: C. Chen: None.

Poster

529. Social Cognition: Behavior, Neural Basis and Pharmacology

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 529.03/Y21

Topic: F.01. Human Cognition and Behavior

Title: Actor-observer bias in moral evaluation: an fMRI

Authors: *Y.-J. CHEN;
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Abstract: Morality is a fundamental component of human cultures and has been defined as prescriptive norms regarding how people should treat one another, including concepts such as
justice, fairness, and rights. Actor-observer bias explains the errors that one makes when forming attributions about the behavior of others. When people judge their own behavior, and they are the actor, they are more likely to attribute their actions to the particular situation than to a generalization about their personality. Yet when an observer is explaining the behavior of another person, they are more likely to attribute this behavior to the actors’ overall disposition rather than to situational factors. Many studies have explored the influence of moral evaluations on judgments about the intentionality of actions. Using fMRI, the current study examined the extent to which actor-observer bias affect behavioral ratings of praise and blame and how they modulate the online neural response when participants evaluate morally laden (good and bad) everyday actions. The activity in the amygdala, anterior insula, ventromedial, and dorsolateral prefrontal cortex (DIPFC) was increased to perceived agency in spite of comparable subjective ratings. The effective connectivity between amygdala and dIPFC was reduced. The activity in the temporoparietal junction predicted utilitarian responses to evitable dilemmas. The findings demonstrate acute effect of testosterone on neural responses associated with moral judgments. Administering a single dose of testosterone can modulate the capacity of emotional regulation and perspective taking, which, in turn, heightens reactivity to social provocation and to make utilitarian judgments.

Disclosures: Y. Chen: None.

Poster

529. Social Cognition: Behavior, Neural Basis and Pharmacology

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Program#/Poster#: 529.04/Y22

Topic: F.01. Human Cognition and Behavior

Support: NRF-2012-S1A3-A203375

Title: Oxytocin differentially affects moral judgment of permissibility and punishment

Abstract: People consider both intention and outcome of an actor’s behavior when making moral judgment. A growing body of evidence suggests that these two types of information are processed by separate neural systems (Treadway et al., 2014; Yu, Li & Zhou, 2015) and the extent to which the judgment is affected by each type of information depends on the specific type of moral decision being made (Cushman, 2008). The present study aims to investigate the effect of intra-nasally administered oxytocin (OXT), the evolutionarily preserved neuropeptide that is known to influence a wide range of prosocial behaviors (Hurlmann et al., 2010), on the way that intention and outcome affect moral judgments of permissibility and punishment. In a between subject, double blind, and placebo-controlled design experiment, 38 male participants were randomly divided into two groups and self-administered 40 IU/ml of oxytocin (N=22) or placebo (N=16) using intranasal sprays. The main task design and stimuli were adopted from Young and Saxe (2008): Participants were required to read a series of short scenarios presented through computer screen and indicated whether the action of the protagonist depicted in the scenario was 1) morally permissible and 2) punishable on the 7-point likert scale. The valences of intention and outcome are independently manipulated so that 4 unique combinations of a protagonist’s intention (neutral vs. negative) and outcome (neutral vs. negative) were created for each scenario. In order to investigate the effect of OXT on two types of moral judgments, two separate 2(Group) x 2(Intention) x 2(Outcome) mixed ANOVA were performed on the behavioral ratings and reaction times(RT) for each combination of intention and outcome in both tasks. The analysis showed no significant OXT-related effects in overall rating patterns in both tasks. However, OXT does seem to influence RT and have a mitigating effect in the punishment judgment task: Participants with OXT administration than those in the placebo group were generally slower to respond, and tended to give more lenient punishment ratings to the sets of intention-outcome combinations that fall in the intermediate level of emotional impact or perceived “severity,” as opposed to those with low or high severity. This pattern of effect in behavioral rating is only present in punishment judgment. These results are consistent with the previous findings that OXT effect could be regulated by various contextual factors (Bartz et al., 2011), and suggest that social approach tendency induced by OXT administration may run counter to the decision mechanism involved in punishment judgment.


Poster

529. Social Cognition: Behavior, Neural Basis and Pharmacology

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 529.05/Y23
Abstract: Oxytocin (OT) plays a role in social cognition, but the specific nature of this role in the human brain, and the extent to which individual differences may contribute to variation in the response to intranasal OT, are still under investigation. The current double-blind, placebo-controlled study involved 30 female subjects. At visit 1, all subjects received intranasal placebo. At visit 2, half of the subjects were randomly assigned to receive intranasal OT, while the other half received placebo again. All subjects were not on hormonal birth control and experienced natural regular menstrual cycles, and both visits occurred approximately 30 days apart during the luteal phase of the menstrual cycle. Measures included a battery of psychological and behavioral tests as well as a neuroimaging session. In an fMRI task, subjects viewed cartoons of moving shapes, some of which could be interpreted as positive- or negative-valence social interactions, and some of which simply depicted random movement. Subjects responded with button presses to answer questions about the shapes’ social relationships or physical characteristics. After scanning, subjects re-watched the videos and provided free-response descriptions. fMRI results indicate that intranasal OT increased activation in regions of the brain associated with social and affective processing during trials in which the shapes’ movements were perceived as social interactions. These regions included ventromedial and orbitofrontal cortex, the superior temporal sulcus and lateral temporal regions, amygdala, inferior frontal gyrus, and inferior parietal cortex. Interestingly, many of these regions, particularly visual sensory regions, also showed some degree of OT-induced increased activation during trials where shapes merely moved randomly around the screen. We also examined the relationship between individual psychological variation, variation in brain organization, and response to OT. Together, these results expand our understanding of the effects of intranasal OT, provide information about the extent to which individual differences may mediate OT’s effects, and raise new questions about the role of endogenous OT in social and non-social cognition.

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**Abstract:** Human social behavior is largely based on the interpretation of actions of others during social interaction. Cooperative interaction promotes and strengthens social bonding with prosocial behavior, associated with feelings such as friendship and liking, whereas non-cooperative interaction leads to rejection or even punishment associated with feelings of disliking and hate. Distinguishing between cooperators and non-cooperators involves theory of mind: the ability to infer the mental states of others. Here we examined the similarities and differences of the neural responses while inferring mental states of cooperators and non-cooperators. Thirty-four volunteers participated in a social interaction (a modified version of the Dictator game) with two confederates: one used a cooperation strategy (Coop), and the other a non-cooperation strategy (NoCoop). As a result of experiencing the confederate’s strategies, the participants increased the amount of money offered to the Coop and decrease it to the NoCoop. In a subsequent fMRI session participants were asked to think about the confederates feelings in situations with negative or positive emotional valence. Results revealed that all conditions activated the theory of mind network (i.e. temporoparietal junction, precuneus, medial prefrontal cortex). However, the response to the Coop and NoCoop situations resulted in distinct activations; negative situations lead to increased activity in the left prefrontal cortex for Coop, and bilateral prefrontal cortex as well as the right middle temporal gyrus for NoCoop. A final analysis showed that confederate liking ratings correlated with activity in social and emotional areas for Coop in positive situations (left temporoparietal junction, right temporal pole, and bilateral amygdala). While for NoCoop in negative situations correlated with incentive-based learning areas (right caudate and putamen, and left accumbens). Both conditions could indicate positive affect in the participants. These results suggest that thinking about non-cooperators mental states demand more cognitive control and semantic processing. The results also suggest that the degree of affection developed to the cooperators involve mechanisms for sharing mental and emotional states. In contrast, when dealing with non-cooperators, mechanisms related to the learning of social-reward signals are used instead.

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Poster

529. Social Cognition: Behavior, Neural Basis and Pharmacology

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

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Topic: F.01. Human Cognition and Behavior

Support: ERC NeuroCoDec 309865

Title: Advisors adapt their confidence reports strategy to increase their future influence

Authors: *U. HERTZ1,2, B. BAHRAMI2;
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Abstract: The exchange, announcement, of private information and opinions, which finally inspire action, is at the heart of human social interaction. Flow of information from the most informed party to the least informed one is not as straightforward as it seems. The transmission of information is strictly linked with reputation, influence, and personal traits and inclinations that could impact it. Recent studies have focused on the client or advisee point of view, developing computational models for tracking the reliability and expertise of advisers (Behrens et al, 2008; Boorman et al, 2013). From an adviser point of view, influence has a fundamental role on the amount and type of information that will be conveyed (Bayarri and DeGroot, 1989). In fact, the communication choices have strong effects on reputation, trust and credibility. The wrong choices could lead to an instant invalidation of all the previous efforts and achievements. We developed a novel task to evaluate the strategies that an adviser utilizes when transmitting Information. On a trial by trial basis the participant has access to a probability of reward being in one of two locations. He is then asked to advise a client about the location of the reward using a confidence scale. Another adviser also provides confidence report to the client, available to the participant. The client makes a decision, and according to its outcome and the adviser’s confidence reports updates the influence weights assigned to each adviser. The task was published online in Amazon Mechanical Turk. 58 participants completed the task, 31 males (age range 21-56) and 27 females (age range 23-55). Our results show that participants tend to change their confidence report policy according to their influence on the client. When the client preferred the other adviser participants reported confidence was higher compared to when the client was following them. When their influence was high and the client was following their advice participants’ reports tended to be better calibrated with evidence, rendering them more reliable. However, when the client preferred the other adviser their reports became more extreme and less reliable. The changes in report policy with social influence were observed even when individual differences in perceptual accuracy and confidence reports bias were accounted for.
Confirming the predictions of the theoretical model (Bayarri and DeGroot, 1989), these results show that people communicate in a way that takes into account their current social status and the effect of their reports on their future influence.

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Poster
529. Social Cognition: Behavior, Neural Basis and Pharmacology

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Topic: F.01. Human Cognition and Behavior

Support: NIMH Intramural Research Program

Title: Extrapolation of social information to physically similar individuals contributes to stereotyping

Authors: *B. A. LEVY*, C. I. BAKER;
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Abstract: Previous evidence demonstrates that social evaluations of a face can transfer to morphed versions of the same face even when the transformed face is perceived as a new identity (Todorov et al., 2010). The current study investigated whether this similarity-driven generalization of social information functions at a group level to induce stereotyping. Morphing software was used to create three different groups of nine faces with high degrees of within-group similarity. In the first study, a subset of individuals from each group appeared in a learning task that took the form of an investment game. The individuals acted as trustees that returned or kept money invested with them by the participant. Individuals from one group returned the investment on 80% of trials while those from the other two groups did so on 50% and 20% of trials, respectively. Participants were not made aware that the faces belonged to different groups. Participants then completed a test phase in which they replayed the game, this time choosing whether to invest with novel individuals from each group while receiving no feedback as to whether each individual returned or kept the investment. Paired t-tests showed that the behavior of the individuals seen during the learning phase substantially influenced the frequency with which participants chose to invest with physically similar individuals in the test phase, as well as explicit trustworthiness ratings of those individuals. Notably, this effect occurred despite a complete lack of feedback that could be used to judge the trustworthiness of the novel individuals seen in the test phase, suggesting that information about a subset of individuals is
readily utilized to create group-level stereotypes that bias evaluations of perceptually similar individuals. In the second study, participants completed an alternate version of the paradigm in which the learning phase consisted of a guessing game where individuals from each of the three groups were paired with monetary reward 80%, 50%, or 20% of the time, respectively. This change eliminated the relationship between the behavior of the individuals seen in the learning phase and the frequency with which participants chose to invest with physically similar individuals during the test phase, suggesting that the results of the first study occurred due to generalization of a trustworthiness stereotype rather than simply a learned association between certain faces and reward.

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**Poster**

529. Social Cognition: Behavior, Neural Basis and Pharmacology

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 529.09/Y27

**Topic:** F.01. Human Cognition and Behavior

**Title:** Visual preference for images of primate faces in non-human primates

**Authors:** D. DZIOBEK¹, S. ZHANG², J. ASHE³, *X. LU⁴,⁵


**Abstract:** Autism is a devastating neurological disorder of unknown cause, unclear pathogenesis and without an effective treatment. The defining feature of autism is a profound deficit in social behavior including a lack of interest in social bonding, particularly, reduced eye contact. Rodent models of autism may not accurately recapitulate the social deficits in primate behavior characteristic of the disease. Non-human primates, however, have a highly developed sense of social cognition, and ethological studies of these animals have shown that they have more interest in information about social interactions than environmental cues that are critical for survival. We tested the extent to which social cognition determined behavioral choices in non-human primates. Specifically, we examined the preference of monkeys for images of faces relative to images of other familiar and unfamiliar inanimate objects. In an effort to characterize the strength of natural social behaviors in non-human primates, we studied their preferences for different classes of images including: (i) familiar and unfamiliar non-food inanimate objects, (ii) familiar food, (iii) and familiar and unfamiliar human and monkey faces. In the behavioral task,
two animal subjects were required to indicate their preference for one of two simultaneously presented visual images by making a saccade to that image after which they received a liquid reward. In the majority of trails, the images were from different classes. When presented with two neutral inanimate images, the monkeys showed no preference (p<0.0001, binomial distribution fit). However, when images of a face were paired with a neutral object or a familiar food, the monkeys preferred the face in more than 80% of trials (p<0.0001, binomial distribution fit). There was no difference in preference for familiar or unfamiliar faces. In addition to their preference, the animals had shorter performance times toward faces (p<0.01, Wilcoxon rank sum). Our data suggest the preference of non-human primates for faces is driven by considerations of social cognition that are so prominent in all primates. The overwhelming preference for images of faces was maintained even when paired with images of preferred foods. We believe that this natural tendency to prefer images of other primates could be used as an assay to probe autistic behavior in monkey models of autism. Eliciting changes in this preference for faces, inactivating or stimulating specific brain regions in non-human primates using pharmacological or electrical methods would provide much needed insights into which neural circuits and processes may be perturbed in human cases of autism.

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Poster

529. Social Cognition: Behavior, Neural Basis and Pharmacology

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Topic: F.01. Human Cognition and Behavior

Support: Max Planck Society

Wellcome Trust

Title: The role of oxytocin in altruism and emotional responses to inequality

Authors: *R. B. Rutledge, A. O. De Berker, S. Espenhahn, R. J. Dolan; Univ. Col. London, London, United Kingdom

Abstract: Social comparison is an important factor in subjective well-being and many studies suggest that relative wealth is a major determinant of life satisfaction. We developed a social decision-making task which allowed us to quantify the impact of inequality aversion on momentary subjective well-being or happiness. We found that well-being reflected not only
rewards received by the participant, but also rewards received by their partner. Unequal outcomes, whether advantageous or disadvantageous ('guilt' or 'envy', respectively), reduced average well-being. As the neuropeptide oxytocin plays a role in a range of social behaviors, including altruism, we conjectured that this might partly depend on inequality aversion. Thus we repeated this experiment in a double-blind placebo-controlled design in which participants received intranasal oxytocin in one session and placebo in the other session. Although well-being was affected by both advantageous and disadvantageous inequality, oxytocin selectively reduced the negative impact of disadvantageous inequality, leaving unaltered the effect of advantageous inequality on well-being. Oxytocin administration in the same subjects increased generosity in a separate social value orientation task. We discuss these findings in light of oxytocin's role in prosocial behaviors.


Poster

529. Social Cognition: Behavior, Neural Basis and Pharmacology

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 529.11/Y29

Topic: F.01. Human Cognition and Behavior

Support: NIH Grant 5R21HD076164

Title: Dopamine mediates human maternal bonding. A behavioral PET-fMRI study

Authors: *S. ATZIL*, C. CATANA, B. C. DICKERSON, R. FELDMAN, J. HOOKER, L. F. BARRETT; 
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Abstract: Introduction- In rodents, the dopaminergic circuit is involved in social affiliation, specifically in the context of maternal bonding. Additionally, individual differences in bonding behaviors in rats are related to differential mesolimbic dopaminergic activity (Champagne et al. 2004). In humans, a dopaminergic mechanism for social affiliation has yet to be evaluated. We hypothesized that human maternal bonding involves dopaminergic reward circuitry. We further hypothesized that individual differences in human maternal bonding depends on this dopaminergic reward circuitry. To test our hypotheses, we applied for the first time simultaneous fMRI-PET imaging to mothers while watching videos of their infants. Additionally, we tested
whether bonding behavior predicted the social reward response. Methods and Results- To evaluate whether mothers secret dopamine in response to their infants, we assessed the binding potential of raclopride, a D2 receptor antagonist radiotracer, while mothers are viewing their own compared to an unfamiliar infant films. We predict that mothers will respond to their own infants with increased dopamine release. In line with our hypothesis, mothers had a significantly higher dopamine release during the own infant film in the left pallidum (t= -5.283, p=0.001), left hippocampus (t= -2.468, p= 0.036) and left amygdala (t= -2.784, p= 0.024). Individual differences in bonding was assessed by behavioral mother-infant synchrony, and used as a predictor for the maternal dopaminergic response. Mothers who are more synchronous with their infants had higher dopamine secretion to their infant in the right pallidum (Rpearson= -0.79, p=0.02). The sensitivity to social reward (assessed by questionnaire) robustly predicted higher selective dopamine secretion to the own infant in the left and right putamen (Rpearson= -0.856, -0.837, p=0.007, 0.009). Discussion- This study demonstrates for the first time that human mothers respond to their infants with dopaminergic secretion, which is consistent with the rodent literature. Moreover, individual differences in bonding are related to distinct dopaminergic pattern, as synchronous mothers have higher dopaminergic reward response to their infant. These preliminary results provide initial proof of concept for the use of PET and specifically D2 radiotracers to study social-affiliation in humans.


Poster

529. Social Cognition: Behavior, Neural Basis and Pharmacology

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 529.12/Y30

Topic: F.01. Human Cognition and Behavior

Support: PIA grant ANR-10-IAIHU- 06
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CONICYT Chile, PCHA, Master 2 y Doctorado
ANR grant ANR-12-SAMA-011
ANR grant ANR-10-CREA-005
Early attention orienting effect and implicit liking effect during an eye gaze cueing task: a magnetoencephalography study

Authors: *N. George*1,2,3,5, S. Dubal6, J. Ulloa5;

1Inst. du Cerveau et de la Moelle Epiniere (ICM), Paris, France; 2Umr 7225, CNRS, Paris, France; 3U1127, Inserm, Paris, France; 4Umr_s 1127, Sorbonne Universités - UPMC Univ. Paris 6, Paris, France; 5Ctr. MEG-EEG, ICM/CNRS/Inserm/UPMC/ENS, Paris, France; 6ICM, CNRS / Inserm / UPMC, Paris, France

Abstract: Social cues can greatly impact our perception of surrounding objects. For instance, eye gaze signals others' current object of attention and potential preferences, and in doing so, it has the power to influence our attentional state and affective evaluation of seen objects. While social influences have been long ago acknowledged, their neural underpinnings have only recently started to be investigated and are still to be fully clarified. Here, we aimed at investigating the modulations of brain activity related to attention orienting and to implicit affective value attributed to visual targets during an eye gaze cueing task. Twenty-six participants participated in this magnetoencephalography (MEG) study. We recorded the neuromagnetic activities evoked by seen targets when participants were engaged in a Posner-like paradigm of attention orienting by gaze. A liking rating task was then used to assess the hedonic value attributed to the targets. Using source localization based on weighted Minimum-Norm Estimate (wMNE), we analyzed the brain responses during the target display period as a function of attention orienting and hedonic evaluation. Our results were twofold. First, we observed faster response times for valid (looked-at) than invalid (not looked-at) targets, reflecting the typical attention orienting effect induced by eye gaze or eye gaze cueing effect. This effect was accompanied by enhanced responses to invalid relative to valid targets between 90 and 400 ms in occipital and superior parietal regions, with responses lateralized to the hemisphere contralateral to the target presentation side. This demonstrates early neural effects of attention induced by eye gaze. Second, although we did not find a reliable influence of eye gaze on liking, we showed that, at the neural level, high- relative to low-liked targets elicited enhanced activation in a right posterior lateral temporal region between 90 and 380 ms during the gaze cueing task. This suggests that there is an early and sustained encoding of the implicit hedonic value of objects. Altogether these results shed new light on the attentional and affective processing of objects in a social context.

Disclosures: N. George: None. S. Dubal: None. J. Ulloa: None.

Poster

529. Social Cognition: Behavior, Neural Basis and Pharmacology

Location: Hall A
ERP evidence of the implicit evaluation of self-relevance for everyday objects

Authors: *G. TRUONG, T. S. T. HUANG, T. C. HANDY;
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Abstract: When made explicit through cueing or instruction, self-relevance has been found to drive attention and increase cognitive processing (Turk et al., 2011; Cunningham et al., 2008). However, there is evidence that attentional capture by self-related stimuli can also occur automatically (Alexopoulos et al., 2012). Given that previous research (Handy et al., 2010) shows that people make hedonic preference judgments about objects rapidly and implicitly, the current study investigated whether people also make rapid and implicit self-relevance judgments when viewing objects. Participants viewed a series of images of everyday objects (e.g., pineapple, football) during a supposed target identification task while electrical brain responses were recorded via EEG. Following this task, participants reviewed all of the object images and selected the ten most personally relevant and ten least personally relevant stimuli. We then compared event-related potentials (ERPs) elicited by these two categories. Both objects selected as most relevant and least relevant demonstrated greater P300 mean amplitudes, relative to the P300s elicited by the average of all objects. Furthermore, the late positive potential (LPP) was greater for the most self-relevant objects relative to least self-relevant objects and all objects. These results suggest that although highly self-relevant and highly non-self-relevant stimuli elicit initial attentional processing, highly self-relevant stimuli receive uniquely additional evaluation even when such evaluation is not essential to present task demands.

Disclosures: G. Truong: None. T.S.T. Huang: None. T.C. Handy: None.

Poster

529. Social Cognition: Behavior, Neural Basis and Pharmacology

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 529.14/Y32

Topic: F.01. Human Cognition and Behavior

Support: KAKENHI 21300196
Title: Brain response to overhearing one's own name: an fMRI study

Authors: *T. NAKANE;
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Abstract: It is reported that the medial prefrontal cortex (mPFC) is involved in hearing one's own name. However, it is not clear yet if mPFC always respond to one's own name regardless of ongoing task. We hypothesized that one should not show mPFC activation when s/he is engaged to the task in which one's own name is irrelevant. Thirty healthy adults volunteered to the study. Stimuli were vocalized names of participant's own (S), repeated other's (R), and variable others' as control (C). During a trial, two names (either S+C, R+C, or C+C) were presented binaurally and simultaneously. Each of the names was followed by high or low short tone burst. In the Name detection session, participants judged whether the presented names contained S or R (i.e. target names) and pressed a button. In the Tone judgement session, participants judged whether the beep was high or low. If mPFC always responds to one’s own name, the contrast S-R should activate mPFC in both Name detection and Tone judgement sessions. The results showed that S-R during Name detection session activated mPFC (Figure 1), but that during Tone judgment session did not (Figure 2), even though the identical sound stimuli were presented. We conclude that association of hearing one’s own name and mPFC activation is contingent.
Disclosures: T. Nakane: None.

Poster

529. Social Cognition: Behavior, Neural Basis and Pharmacology

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 529.15/Y33

Topic: F.01. Human Cognition and Behavior

Support: NIH Grant 5K01MH092601

Title: Know thyself; aberrant neural activity during self-reflective processing of depressed youth with trauma history

Authors: *R. NG1, H. SCOTT2, G. SMYDA3, S. MALONE3, K. QUEVEDO2
1Inst. of Child Develop., Univ. of Minnesota, Twin Cities, Minneapolis, MN; 2Dept. of Psychiatry, 3Univ. of Minnesota, Minneapolis, MN; 4Grad. Sch. of Publ. Hlth., Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Negative self-reflective processing is central to depressogenic cognitions, and a potent predictor of severity/recurrence of depressive disorders. In a similar vein, dysfunction in neural networks that support self-reflective processing (midline cortical structures: MCS) and emotion processing (limbic region) has been observed among those with depressive disorders. Importantly, youth with a history of trauma are at significantly greater risk for treatment-resistant depression relative to depressed non-traumatized peers, perhaps partially due to ancillary abnormal corticolimbic functioning underlying self-processing that is resistant to current treatment approaches. To date, however, there is no research that has explored neurobiological correlates of self-processing in youth with depression and trauma. This investigation is important
to our understanding of self-organization in adolescence, a sensitive window for identity formation, particularly when permeated by trauma exposure. This study examined neural activity during the processing of positive/negative self-descriptors from the self and the mother’s point of view (p.o.v.) in 37 healthy (HC) and 86 depressed adolescents. We applied a mixed block and event-related paradigm to investigate neural activity during processing of positive/negative self descriptors as a function of p.o.v. Among depressed youth, 41 experienced significant trauma (DT) and 45 reported no history of trauma (DC). We hypothesized: 1. DT youth would show greater MCS activation during processing of negative vs. positive self-descriptors compared to both DC and HC; 2. DT adolescents would exhibit more MCS and limbic activity during processing of negative phrases from the mother’s p.o.v than DC and HC youth. Whole-brain and region of interest analyses both supported our predictions. A group x condition interaction indicated that youth with both depression and trauma history showed robust neural activity of MCS and limbic regions during first person p.o.v processing of negative vs. positive self-descriptors compared to DC, while HC youth instead showed heightened activation of MCS for positive vs. negative self-descriptors. Results suggest that higher emotional salience for positive self-information that is known to be protective against psychopathology, is severely altered in depressed traumatized youth. These findings offer novel evidence that self-representations of DT youth are more informed by higher salience of negative self-descriptors from both the self and the mother’s perspective, which in turn, subserves the cognitive vulnerability for chronic depression.


Poster

529. Social Cognition: Behavior, Neural Basis and Pharmacology

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 529.16/Y34

Topic: F.01. Human Cognition and Behavior

Title: Neural responses to heartbeats in the default-mode network encode the self-relatedness of spontaneous thoughts

Authors: *M. BABO REBELO, C. RICHTER, C. TALLON-BAUDRY;
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Abstract: The brain constantly receives and integrates signals from the internal organs (Critchley & Harrison 2013). By creating a dynamic and unified representation of the organism,
these neural maps of visceral activity could generate a basic form of self (Craig 2003, Damasio 2010). The brain monitoring of the heart can be studied via neural responses to heartbeats which have been recorded in the medial prefrontal cortex (Park et al 2014), a visceral center implicated in self-related processing (Northoff et al 2006). Here, we investigate the link between heartbeat-evoked responses (HERs) and the self. We considered the self in two distinct dimensions (Christoff et al 2011): the self as subject - the subject who acts, perceives or feels; and the self as object - the reflective self, when explicitly thinking about oneself. 20 human participants fixated a screen and mind-wandered until a visual stimulus interrupted their spontaneous thoughts at random intervals. Interrupted thoughts were then evaluated by the subject on four continuous scales: the degree of involvement of the self as subject (low to high), the object of the thought (self/external), the time (past to future) and valence. We recorded magnetoencephalographic and cardiac activity to test whether the amplitude of HERs (evoked-responses locked to the T-wave of the cardiac cycle) before the interrupting stimulus reflected the orientation of the ongoing thought, on each scale. HERs significantly differed depending on the involvement of the subject as the subject, the protagonist, of the ongoing thought ([298 327ms] after the T-peak, Monte-Carlo p=0.0397). This difference was localized in the left ventral precuneus (vPC, Monte-Carlo p=0.037). HERs significantly differed depending on the object of thought ([94 169ms] after the T-peak, p=0.0112), in the left ventromedial prefrontal cortex (vmPFC, Monte-Carlo p=0.030). No differences were found for time and valence. We show here that the brain monitoring of visceral signals encodes the self-relatedness of spontaneous thoughts in the default-mode network (DMN), in line with our hypothesis that neural responses to visceral inputs anchor self-related thoughts to an internal reference frame. The vmPFC is a visceral center of the brain (Vogt & Derbyshire 2009) and would be more related to self evaluation and introspection. The vPC is considered a rich club node (van den Heuvel & Sporns, 2013), contributing to the integration of information across resting state networks. Such a central role in brain dynamics fits well with the idea that the vPC is implicated in the most basic form of self.

Disclosures: M. Babo Rebelo: None. C. Richter: None. C. Tallon-Baudry: None.

Poster

530. Pharmacology of Executive Function

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 530.01/Y35

Topic: F.02. Animal Cognition and Behavior

Support: Polish National Science Center Grant SONATA 2012/07/E/NZ3/01785
Title: NMDA receptors in noradrenergic neurons regulate tonic activity of locus coeruleus and facilitate attentional set shifting in mice

Authors: *P. E. CIESLAK*¹, N. LLAMOSAS², T. KOS¹, L. UGEDO², M. TORRECILLA², J. RODRIGUEZ PARKITNA¹;
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Abstract: Noradrenergic (NA) neurons of the locus coeruleus (LC) exhibit tonic and phasic modes of activity, which closely correspond to attentional processing and behavioral performance. Here, by using NR1DbhCre transgenic mouse strain, we tested effects of inactivation of NMDA receptors on LC activity and behavioral performance in tasks requiring selective responding in a stable environment (the go-no/go discrimination task) or flexible responding in a changing environment (the attentional set shifting task, ASST). We found that mutation caused an increase in spontaneous tonic activity of noradrenergic cells in LC, without appreciably affecting their phasic (bursting) activity. On the behavioral level, both control and mutant mice exhibited high accuracy of responding to „go” signals (hit rate) and limited ability to refrain from responding to “no-go” signals (false alarm rate). Detailed analysis revealed that mutant mice had decreased ability to discriminate between “go” and “no-go” signals, which could indicate increased distractibility. However, mutant mice exhibited also facilitated shifting of attention between different types of stimuli (digging media and odors), in the ASST paradigm. These results show that NMDA receptors located in NA cells regulate tonic activity of LC and that selective loss of NMDA receptors facilitate attentional set shifting, possibly by increasing the propensity for exploration.


Poster

530. Pharmacology of Executive Function

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 530.02/Y36

Topic: F.02. Animal Cognition and Behavior

Support: NIH MN093354

Alz Assoc NIRG-11-203107
Nicotinic α4β2 receptor stimulation strengthens both working memory- and attention-related neuronal activity in prefrontal cortex

**Authors:** *M. WANG*, Y. YANG1, Y. SUN2, S. YANG1, L. E. JIN1, V. C. GALVIN1, A. F. T. ARNSTEN1;
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**Abstract:** Acetylcholine (Ach) projections to the primate dorsolateral prefrontal cortex (dlPFC) play a critical role in working memory and attention regulation. Electrophysiological studies in awake behaving monkeys performing a spatial working memory task have found that the dlPFC contains many Delay cells and Fixations cells, which are considered as the cellular basis underlying working memory and sustained attention. Delay cells show spatially tuned, persistent firing across the delay period when the spatial position must be held in working memory, while Fixation cells increase firing at trial onset and sustain firing until the saccadic response. Our previous studies showed that Ach acting at nicotinic α7 receptors (nic-α7R) has an essential influence on the working memory-related firing of Delay neurons in the primate dlPFC, permitting NMDA receptor (NMDAR) actions in glutamate synapses, and rescuing neuronal firing from NMDAR blockade. Here, we report that Ach acting at another nicotinic receptor, the nic-α4β2R, has subtle beneficial effects on Delay cell firing, but far more prominent beneficial influences on dlPFC Fixation cells that relate to sustaining attention across the length of a trial. Furthermore, nic-α4β2R stimulation of Delay cells can rescue the reduction in delay-related firing caused by presenting distractors during the delay period. These data suggest that nic-α4β2R agonists may be useful in the treatment of Attention Deficit Hyperactivity Disorder and related conditions with impaired sustained attention.


**Poster**

530. Pharmacology of Executive Function

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#: 530.03/Y37

**Topic:** F.02. Animal Cognition and Behavior

**Support:** Baylor College of Medicine IDDRC Grant Number 1 U54 HD083092 from the Eunice Kennedy Shriver National Institute of Child Health & Human Development

**Title:** Behavioral phenotyping of mice deficient in CHRNA7
Authors: *J. YIN*\(^1,2\), C. SCHAAF\(^2,1\);
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Abstract: 15q13.3 microdeletion syndrome is a rare genetic disorder caused by a deletion of a segment of chromosome 15. The deletion is commonly 1.5Mb in length and encompasses 6 genes, but patients with small deletions, which only encompass the CHRNA7 gene, have also been reported. The clinical phenotypes associated with this syndrome are variable, but commonly include developmental delay/intellectual disability and impaired social interaction. Other clinically important features include epilepsy, impulsive behavior, aggression, depression, and schizophrenia. A mouse model deficient of CHRNA7 was generated and reported to be grossly normal in growth, anxiety-like behaviors, learning and memory, as well as sensorimotor gating. However, the presence of repetitive behavior, altered social interaction or depression had not been assessed in this mouse model. We tested heterozygous and homozygous Chrna7 mutant mice and their wildtype littermates for repetitive behaviors in self-grooming, holeboard exploration, and marble burying, for social interaction behaviors in the three-chamber paradigm, partition test, social interaction video scoring, and tube test, and depression in the tail suspension test and forced swimming test. The Chrna7 knockout mice showed increased marble burying and self-grooming behaviors depending on gender. In addition, knockout mice of both genders had significantly more social avoidance behaviors compared to heterozygous and wildtype littermates, suggesting an altered social response, as can be seen in human patients as well. Compared to human clinical manifestations in individuals with homozygous or heterozygous 15q13.3 deletion, the phenotypes present in this Chrna7 knockout mouse model are subtle, which raises questions about the potential contribution of other genes in the 15q13.3 locus, or possibly the presence of compensatory mechanisms in mouse which can not fully compensate for the loss of CHRNA7 in humans.

Disclosures: J. Yin: None. C. Schaaf: None.

Poster

530. Pharmacology of Executive Function

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

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Topic: F.02. Animal Cognition and Behavior

Support: Center for Behavioral Brain Research

DFG: SFB 779
Title: Optogenetic silencing of locus coeruleus activity in mice impairs cognitive flexibility in an attentional set-shifting task

Authors: K. JANITZKY1,2, *M. T. LIPPERT3, A. ENDELHORN4, J. TEGTMEIER5, J. GOLDSCHMIDT1,6, H.-J. HEINZE2,5,6, F. W. OHL1,3,6;
1LIN Magdeburg, Magdeburg, Germany; 2Dept. of Neurol., 3Inst. of Biol., Otto-von-Guericke Univ., Magdeburg, Germany; 4SPL, 5Dept. Behavioral Neurol., Leibniz Inst. for Neurobio., Magdeburg, Germany; 6Ctr. for Behavioral Brain Sci., Magdeburg, Germany

Abstract: The locus coeruleus (LC) is the main source of noradrenergic projections in the brain and essential for attention-dependent cognitive processes. Here we used unilateral optogenetic silencing of the LC in an attentional set-shifting task (ASST) to analyze the influence of the LC on prefrontal cortex-dependent functions in mice. In order to reversibly silence the LC, we expressed the halorhodopsin eNpHR3.0 in its noradrenergic cells. We found that yellow-light mediated silencing selectively impaired performance in those phases of the task that most critically rely on orbitofrontal and prefrontal function. Both reversal learning and attentional set-shifting were impaired, suggesting a general effect of LC-silencing on cognitive flexibility. In contrast, silencing did not have a measurable effect on the phases of the task that are less dependent on cognitive flexibility (e.g. compound discrimination and intra-dimensional shifts). These findings suggest a modulatory influence of the LC on cognitive flexibility, likely mediated through both prefrontal and orbitofrontal networks.


Poster

530. Pharmacology of Executive Function

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 530.05/Y39

Topic: F.02. Animal Cognition and Behavior

Title: The effects of histone deacetylase inhibitors treatment on executive function and prefrontal cortex in adolescent rats

Authors: *J. A. MCGAUGHY1, G. WELCH2, F. M. VASSOLER3, E. M. BYRNES3;
1Dept Psych, Univ. New Hampshire, Durham, NH; 2Univ. of New Hampshire, Durham, NH; 3Biomed. Science, Section of Neurosci., Cummings Sch. of Vet. Med. Tufts Univ., North Grafton, MA
Abstract: In preclinical models, histone deacetylase inhibitors (HDACi) have been proposed as a potential class of cognitive enhancers for neurodevelopmental, neuropsychiatric, and neurodegenerative diseases associated with deficits in learning and memory. To date, the effects of these potential cognitive enhancers on executive function, specifically selective attention, during normal neurodevelopment remain unknown. Adolescent rats have poor impulse control and are less cognitively flexible than adults as shown by both a susceptibility to distraction and increased cognitive rigidity in tests that require attentional set shifts. Recent work from our lab supports the hypothesis that high densities of norepinephrine transporters in the prefrontal cortices during adolescence leads to immaturities in attentional set shifting that are mitigated by the administration of selective norepinephrine reuptake inhibitors. HDACi increase transcription of the norepinephrine transport (NET) protein, which is hypothesized to further decrease extracellular norepinephrine and increase cognitive rigidity in adolescent rats. The current study investigated the effects of HDACi on the performance of adolescent rats in an intradimensional/extradimensional attentional set shifting task (ID/ED). Adolescent animals treated with the HDACi, sodium butyrate (0.0, 0.6 or 1.2 g/kg/ml) showed differential effects on performance that varied based on cognitive demands. In a separate cohort, western blotting was used to assess HDAC inhibition in prefrontal subregions critical to executive function as well as the effect of this drug on adolescent cortical NET levels. These data will provide additional information regarding the potential use of HDACi as cognitive enhancers during a key developmental period and will provide additional information on the role of specific subregions of the prefrontal cortices in moderating these effects.

Disclosures: J.A. McGaughy: A. Employment/Salary (full or part-time); University of New Hampshire. G. Welch: None. F.M. Vassoler: None. E.M. Byrnes: None.

Poster

530. Pharmacology of Executive Function

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 530.06/Y40

Topic: F.02. Animal Cognition and Behavior

Support: NSERC

FRQS

FRQNT
Title: Increased impulsive choice following repeated exposure to methylphenidate during early adolescence but not during adulthood

Authors: *Z. Abbas¹, A. Sweet¹, E. Afrand¹, G. Hernandez², A. Arvanitogiannis¹; ¹Psychology, Concordia Univ., Montreal, QC, Canada; ²Psychology, Univ. de Montréal, Montreal, QC, Canada

Abstract: It is known that the psychostimulant methylphenidate (commonly known as Ritalin) improves measures of self-control and impulsive choice. What is not sufficiently understood is how repeated treatment with methylphenidate affects impulsive choice in the long run and whether exposure to methylphenidate at different periods of life produces similar or different long-term effects on impulsivity. Here we used an animal model for impulsive choice to explore these issues. First, we examined whether giving methylphenidate through early adolescence would affect delay aversion, an operational measure of impulsivity, later in adulthood. We then tested whether equivalent long-term effects would be observed if exposure to the drug occurred during adulthood. Delay aversion refers simply to a high preference for immediate smaller rewards over delayed larger rewards. Starting on postnatal day 25 or postnatal day 60, male rats received one of four doses of methylphenidate (0, 2, 4 or 8 mg/kg; ip) for ten consecutive days. Twenty-five days later, all rats were trained in operant conditioning chambers to choose between a lever that produced a small (one food pellet) immediate reward and a lever that produced a large (four food pellets) delayed reward across a range of delays (from 0.1-s to 63-s). After performance stabilized, all groups showed the expected orderly decrease in preference for the larger reward as the delay to obtain it increased. Importantly, analyses revealed a reduced selection of the delayed larger reward, indicative of increased impulsive choice, in those animals that were treated with lower doses of methylphenidate during early adolescence but not the higher dose. In contrast, no differences were observed in the selection of the delayed larger reward in animals that were treated with various doses of methylphenidate during adulthood. These findings suggest that methylphenidate exposure during different stages of life has disparate effects on impulsive choice. When given during adolescence, treatment with certain doses of methylphenidate can increase impulsive choice long after the end of treatment; however, the same may not be true when methylphenidate treatment is given during adulthood.

Disclosures: Z. Abbas: None. A. Sweet: None. E. Afrand: None. G. Hernandez: None. A. Arvanitogiannis: None.

Poster

530. Pharmacology of Executive Function

Location: Hall A
Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 530.07/Y41

Topic: F.02. Animal Cognition and Behavior

Title: Prefrontal cortical Neuregulin-ErbB4 modulation of impulsive behavior in rats

Authors: *T. PATTIJ', D. SCHETTERS', M. LOOS', S. SPIJKER', T. J. DE VRIES';
1VU Univ. Med. Ctr., Amsterdam, Netherlands; 2Sylics, Amsterdam, Netherlands; 3VU Univ., Amsterdam, Netherlands

Abstract: Impulse control disturbances are key features of various neuropsychiatric and neurological disorders, such as attention-deficit/hyperactivity disorder, drug addiction, Parkinson’s disease and schizophrenia. Whereas over the last years accumulating evidence has highlighted monoaminergic modulation of the processes underlying impulse control, investigating novel mechanisms beyond monoamines may provide new intervention strategies to ameliorate impulse control disturbances. Recent work has associated the Neuregulin (Nrg)-ErbB4 pathway with several neuropsychiatric diseases, as well as indicated its involvement in preclinical murine measures of impulse control [Loos et al (2014) Biol Psychiatry 76]. The aim of the present study was to further investigate the role of this Nrg-ErbB4-dependent mechanism in impulsive action in rats. To this end, a group of rats was trained in the 5-choice serial reaction time task (5-CSRTT), an operant paradigm that provide measures of visuospatial attention and inhibitory control processes. Upon stable baseline performance, during test sessions the ErbB4 tyrosine kinase receptor inhibitor JNJ-28871063 (JNJ) was intracranially infused into the medioprefrontal cortex. Results showed that JNJ dose-dependently improved measures of impulsive action. Importantly, other measures in the 5-CSRTT reflecting visuospatial attention or aspects of motivational behavior were not altered by JNJ. In conclusion, the present data further strengthen a role for the Nrg-ErbB4 pathway in the prefrontal cortex in cognitive functioning, and in particular point towards involvement in the processes underlying impulse control.


Poster

530. Pharmacology of Executive Function

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 530.08/Y42
**Topic**: F.02. Animal Cognition and Behavior

**Support**: NIDA Grant PO1 DA031656

**Title**: Cortico-striatal interactions mediating sustained attention performance: Simultaneous high-temporal resolution/multi-analyte microdialysis in prefrontal cortex and striatum

**Authors**: *Y. KIM¹, O. S. MABROUK², M. SARTER³; ¹Dept. of Psychology, ²Dept. of Chem. and Dept. of Pharmacol., ³Dept. of Psychology and Neurosci. Program, Univ. of Michigan, Ann Arbor, MI

**Abstract**: Cholinergic activity in the cortex is necessary for attentional performance. Specifically, prior studies demonstrated that removal of cholinergic input impairs the detection of cues (e.g., McGaughy et al., 1966) and that optogenetic enhancement of cholinergic activity enhances hit rates (Gritton et al., 2015). Moreover, prior studies using conventional microdialysis methods indicated attentional task-associated increases in cholinergic activity during task performance and that the presentation of distractors augmented cholinergic activity even while performance suffered (St. Peters et al., 2011). Conversely, we found that poor attentional control observed as a trait in sign-tracking rats is associated with relatively low levels of cortical cholinergic activity (Paolone et al. 2013). The collective evidence from these studies supports the hypothesis that levels of cholinergic neuromodulation in the cortex mediate the top-down control of attention, and that variation of cholinergic neuromodulation specifically impact the selection of cues for cue-oriented behavior. However, the selection of cues and the sequencing and execution of cue-associated responses have also been attributed to striatal circuits. We therefore implanted microdialysis probes into the medial prefrontal cortex as well as into the dorsomedial striatal projection region of this cortex. We collected dialysates from both regions in rats performing a sustained attention task involving the reporting of the presence and absence of cues. Moreover, we used benzoyl chloride derivatization and HPLC-mass spectrometry (Song et al., 2011, 2012) to simultaneously determine 16 neurotransmitters and metabolites from minute-based dialysis collections (High-Temporal Resolution/Multi-Analyte Microdialysis; HTRMAM). Results indicate complex correlations and de-correlations between analyte levels across the two brain regions, over time and in response to distractor manipulations. Moreover, results from sign-tracking versus goal-tracking rats suggest that in addition to attenuated levels of cholinergic neuromodulation in the cortex, relatively weaker fronto-striatal co-release patterns are associated with their attentional vulnerabilities. The use of HTRMAM coupled with multi-site dialysis allows a relatively fine-grained, comprehensive characterization of coordinated and dissociated activation patterns across multiple neuronal systems mediating complex behaviors.

**Disclosures**: Y. Kim: None. O.S. Mabrouk: None. M. Sarter: None.
Title: Leptin and lepRb neurons in the medial prefrontal cortex facilitate cognitive flexibility

Authors: *H. ZHANG, X.-Y. LU; UTHSCSA, San Antonio, TX

Abstract: Dysfunction of the medial prefrontal cortex (mPFC) is a hallmark of many psychiatric disorders, impairing executive function such as cognitive flexibility and working memory. Leptin, an adipokine, has been demonstrated to regulate mood and cognitive function. The leptin receptor, LepRb, is expressed in the mPFC; however, the involvement of leptin and LepRb neurons in the mPFC in cognitive flexibility has not been investigated. In this study, an attention set shifting test (ASST) was used to assess the effects of leptin and its target neurons in the mPFC on cognitive flexibility. Mice were injected with different doses of leptin (0, 0.1, 1.0 mg/kg, i.p.) after being trained to dig for food (training session). The test session included seven discrimination, reversal and shift tasks given in a specific sequence. We found that leptin selectively improved simple discrimination, compound discrimination reversal and extradimensional shift. Deletion of LepRb in the mPFC caused no change in cognitive flexibility but abolished the effect of leptin. The extra-dimensional shift was enhanced following acute stimulation of LepRb neurons in the mPFC and impaired after acute inhibition of LepRb neurons in this area using DREADD technology. These results suggest that leptin and LepRb neurons in the mPFC are critical for the regulation of cognitive flexibility.

Disclosures: H. Zhang: None. X. Lu: None.
Title: Alpha-2A noradrenergic activation improves behavioral flexibility during feature-based reversal learning

Authors: *A. HASSANI, M. OEMISCH, M. BALSARRAS, S. WESTENDORFF, T. WOMELSDORF;
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Abstract: The alpha2a-noradrenergic (a2a-NE) agonist Guanfacine is a recently approved drug prescribed for attention-deficit-hyperactivity-disorder amongst others. It targets postsynaptic a2a-NE receptors at pyramidal cell spines where it enhances synaptic efficacy independent of locus coeruleus mediated presynaptic NE modulation. Due to its specificity, it provides a unique opportunity to study the role of cortical noradrenergic stimulation in attentional control. Previously, Guanfacine has been found to improve working memory by enhancing maintenance of task relevant information, and more controversially, to improve attention allocation by reducing distractibility to task irrelevant stimuli. It is unclear if Guanfacine related improvement to attention is solely based on working memory enhancement. In order to elucidate Guanfacine’s actions on attentional performance, we trained a rhesus monkey in a feature-based reversal-learning task in which two simultaneously presented stimuli of different color were alternately associated with reward. Stimulus motion direction informed the monkey of the choice direction. Stimulus dimming served as the cue to initiate a choice and occurred either separately or simultaneously, thereby inducing less or more stimulus conflict, respectively. Prior to task performance, 0.075mg/kg Guanfacine were administered intramuscularly. First, we found that Guanfacine led to an increased number of rewarded trials and an increased proportion of rewarded over error trials. Secondly, Guanfacine decreased overall distractibility, defined as less error saccades towards stimuli. This reduction in distractibility was most apparent when stimuli dimmed simultaneously, requiring higher conflict resolution. Lastly, we found a trend for reduced perseverative errors during reversal learning. We applied a reinforcement-learning model to quantify the role of reward feedback, decision noise, and working memory decay to explain performance. The model indicated that Guanfacine mediated performance showed enhanced sensitivity to positive feedback and reduced decision noise, but had no influence on short-term memory decay of color-reward associations. Our results indicate that Guanfacine enhances behavioral flexibility along 3 separable routes: by making behavior more reliable and persistent, by improving resistance to interference, and by enhancing sensitivity to reward which may explain reduced perseverative errors. These findings show that feature-based reversal learning in monkeys can serve as a versatile animal model to understand neuronal origins of attention deficits in neuropsychiatric conditions.
Title: Cortical afferents to rat locus coeruleus and pericoeruleus: Implications for optimal behavioral performance

Authors: *H. BOWREY, M. H. JAMES, M. D. REIDY, G. ASTON-JONES; Brain Hlth. Inst., Rutgers Univ., Piscataway, NJ

Abstract: Introduction: According to the Adaptive Gain Theory (AGT; Aston-Jones and Cohen, 2005), the locus coeruleus-norepinephrine (LC-NE) system plays an important role in optimizing behavioral performance. Both tonic and phasic LC-NE neuronal activities vary significantly in relation to task performance. For example, during accurate task performance reflecting focused attentiveness, LC neurons fire tonically at a moderate rate and exhibit selective, phasic responses associated with task-related decisions. In contrast, poor performance reflecting task disengagement is associated with increased tonic activity and an absence of phasic activation in LC-NE neurons. The AGT proposes that descending cortical projections to LC may assist in determining transitions between phasic and tonic modes of LC-NE activity. In particular, the orbitofrontal cortex (OFC) and anterior cingulate cortex (ACC) have been highlighted as potential modulators of LC activity due to their critical roles in evaluating rewards and costs. Although a small number of studies have reported the presence of cortical inputs to LC, the topography of LC innervation from OFC and ACC remains to be extensively characterised. Further, the selectivity of cortical input to the peri-LC dendritic region remains unclear.

Methods: Male Sprague-Dawley rats received injections of cholera-toxin B (CTb) retrograde tracer (20nl) unilaterally into either the nuclear core of LC or the peri-LC dendritic area under electrophysiological guidance. At least seven days later, animals were deeply anesthetized and perfused with fixative to visualize the CTb tracer. Brains were sectioned into 25-μm thick sections and stained for both CTb and tyrosine hydroxylase (TH). Retrogradely labelled cells...
were then examined and quantified in various cortical areas (Bregma +4.20 to +2.52), including prefrontal cortex (PL) and infralimbic cortex (IL), anterior cingulate cortex (ACC) and orbital frontal cortex (OFC). Results: All injection sites were confined to the LC/peri-LC areas. Consistent with previous reports, retrogradely labelled cortical cells was observed in IL. In all cases, retrogradely labelled cells were also observed in the PL and ACC. Occasional retrogradely labelled cells were also observed in ventral and lateral OFC regions. Conclusions: Consistent with the hypothesis that OFC and ACC may modulate LC activity, neurons in these regions innervate the LC and peri-LC regions. Studies are ongoing utilising anterograde tracing to characterize the topography of these inputs into LC/peri-LC regions.


Poster

530. Pharmacology of Executive Function

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 530.12/Z2

Topic: F.02. Animal Cognition and Behavior

Support: P50 HD055751

Title: 5HT2A receptor blockade in the orbitofrontal cortex does not attenuate repetitive behaviors in the BTBR mouse

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Abstract: Previous studies have found that individuals diagnosed with autism spectrum disorder (ASD) display impairments in behavioral flexibility as evidenced by a probabilistic reversal learning deficit. This learning deficit is driven by an inability to maintain a new choice pattern, demonstrated by an increase in regressive type errors. Similarly, the BTBR T+Itpr3tf/J (BTBR) mouse model of ASD shows a similar deficit on probabilistic reversal learning that is also due to a specific increase in regressive errors when compared to B6 mice. Many previous studies have implicated the orbitofrontal cortex (OFC) in behavioral flexibility. We recently demonstrated that acute systemic treatment with M100907, a 5HT2A receptor antagonist, attenuates probabilistic reversal learning in the BTBR mouse. The current experiment investigated whether direct infusion of M100907 into the ventral orbitofrontal cortex attenuates a probabilistic reversal
learning deficit and repetitive grooming in BTBR mice. Mice were tested in a spatial
discrimination task using a 80/20 probabilistic reinforcement procedure. In the spatial
discrimination, mice were tested on acquisition and reversal learning across two consecutive
days. Mice learned to obtain a cereal reinforcement from the “correct” spatial location
(reinforced on 80% of trials) compared with the “incorrect” spatial location (reinforced on 20% of trials). The learning criterion in both phases was choosing the ‘correct’ location on 6 consecutive trials. For grooming behavior, mice were individually placed in a clear plastic cage and the cumulative time spent grooming all body regions across 10 min was recorded. Five minutes prior to the reversal learning phase or repetitive grooming test, mice received a bilateral infusion of vehicle, 0.2µg or 0.6µg M100907 into the ventral orbitofrontal cortex. BTBR mice were not impaired on initial learning of the spatial discrimination compared to that of B6 mice, similar to previous findings. Vehicle-treated BTBR mice were impaired on probabilistic reversal learning compared to that of vehicle-treated B6 mice. Neither the 0.2µg or 0.6µg doses of M100907 into the ventral orbitofrontal cortex attenuated the reversal learning deficit in BTBR mice, although both doses led to a significant increase in perseverative errors. In addition, M100907 treatment did not attenuate repetitive grooming in BTBR mice. These findings suggest that altered 5HT2A receptor functioning in the ventral OFC may not be responsible for behavioral inflexibility and other repetitive behaviors in BTBR mice.


Poster

530. Pharmacology of Executive Function

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 530.13/Z3

Topic: F.02. Animal Cognition and Behavior

Title: Serotonin 5HT1A receptor blockade potentiates impulsive effects of a cannabinoid CB1 receptor inverse agonist, but not a neutral antagonist in rats: Possible relevance for pharmaceutical safety

Authors: J. E. JAGIELO-MILLER1, E. S. PLYLER1, T. M. PROPER1, F. M. MYERS1, C. M. LUSKIN1, M. C. NORMANN1, K. VEMURI2, A. MAKRIYANNIS2, *P. J. MCLAUGHLIN1; 1Dept. of Psychology, Edinboro Univ. of Pennsylvania, Edinboro, PA; 2Ctr. for Drug Discovery, Northeastern Univ., Boston, MA
Abstract: CB1 inverse agonists, such as rimonabant, failed to receive FDA approval due to psychiatric side effects, including suicidal depression and anxiety, in a minority of clinical trial participants. This may be partly due to a lack of valid preclinical models of such effects, as well as identifying features of vulnerable subpopulations that can be modeled in preclinical studies. Impulsivity is often a component of suicidal behavior; moreover, dysregulation of the serotonin 5HT1A receptor may play a role in suicide, depression, and other disorders, and has been shown to produce impulsivity in animals on tasks such as Fixed Consecutive Number (FCN). However, interpretations of impulsivity in tasks based on timing of intervals or counting of responses may be confounded with effects on cognitive processes needed to complete the task. Therefore, in the present study, impulsivity was assessed using a novel Variable Consecutive Number task with a discriminative stimulus (VCN-SD), in which male rats were reinforced for pressing a lever only after a criterion number of responses on another lever, which retracted for 2.6 s after each response. The criterion randomly varied between 12, 15, 18, 21, and 24 responses on a per-trial basis, and was signaled by a tone, eliminating counting- or timing-based strategies. To assess whether interrupted communication at the 5HT1A receptor produces vulnerability to negative effects of CB1 inverse agonism, the CB1 inverse agonist AM 251 was administered systemically, first alone, and then following pretreatment with the 5HT1A antagonist WAY 100,635 (WAY). It was found that AM 251 per se did not decrease accuracy (percentage of chains that reached criterion) at doses up to 8.0 mg/kg. However, following pretreatment with either 0.1 or 0.3 mg/kg WAY, which were also ineffective per se, 4.0 mg/kg AM 251 substantially impaired accuracy. Moreover, dose condition interacted with criterion; that is, effects of the WAY-AM 251 combination were only apparent at longer criterion values, strongly implicating an increase in impulsivity. The neutral CB1 antagonist AM 6527 had no effects on accuracy, alone or in combination with WAY. These results clearly indicate impulsivity, often identified as a component of suicide, can be produced by AM 251, during a state of 5HT1A blockade. Future research is needed to determine whether this finding identifies a 5HT1A-related vulnerability to psychiatric symptoms, and to identify others, in clinical populations. It is also suggested that CB1 neutral antagonists such as AM 6527 may have a safer profile in regard to behavioral impairments than CB1 inverse agonists such as AM 251 or rimonabant.


Poster

530. Pharmacology of Executive Function

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM
Abstract: In 1918, McEwen demonstrated that wing defects, caused by mutation or damage, profoundly affect phototaxis in walking *Drosophila* fruit flies (McEwen, 1918). We have recently described experiments showing that flies are constantly monitoring their flying capability and adjust their light/dark preference accordingly (Gorostiza & Brembs, 2014). This discovery revealed that phototaxis, which appears simple and hard-wired, comprises a value-based decision-making stage, negotiating external stimuli with the animal’s internal state. We also discovered that neuronal activity in circuits expressing dopamine and octopamine, respectively, is necessary and sufficient for adjusting light/dark choices in flies. Here, we present data to disentangle the neural circuits involved in this value-based decision-making process. The data were collected in a screen comprising different subpopulations of dopaminergic and octopaminergic neurons, as well as other suitable candidates.


Disclosures: E.A. Gorostiza: None. B. Brembs: None.

Poster

531. Decision Making and Attention: Prefrontal Cortex

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 531.01/Z5

Topic: F.02. Animal Cognition and Behavior

Support: MRC Grant (G0901884)

Title: Revealing prefronto-subcortical circuits in negative emotion regulation using 18F-FDG microPET in marmoset monkeys
Authors: *Y. SHIBA*1,2, T. FRYER3,4, S. SAWIAK5,3,4, Y. HONG3,4, R. TAIT7,6, J. SUCKLING7,8,5, A. SANTANGELO2, A. ROBERTS2,5;

Abstract: Impaired regulation of physiological and cognitive reactivity to potential threat is a core feature of affective disorders including mood and anxiety disorders. However, the neural mechanisms underlying this emotion regulation are poorly understood. It has been suggested that the prefrontal cortex (PFC) plays a crucial role in modulating neural activity in subcortical structures that are responsible for the processing and expression of fear and anxiety. Recent studies from our laboratory showed that an anxious phenotype, including enhanced anxiety to a social stimulus, innate fear and conditioned fear (Agustín-Pavon et al, Biol Psychiat. 2012; Shiba et al 2015 Front Sys Neurosci) was induced by excitotoxic lesions of either the anterior orbitofrontal cortex (antOFC) or ventrolateral PFC (vlPFC) independently. These results raise the question as to whether the antOFC and vlPFC act on common or distinct downstream subcortical targets to down-regulate negative emotion. To address this, the present study combined localised excitotoxic lesions in the PFC of a non-human primate and functional neuroimaging (18F-FDG microPET) with a fear-inducing behavioral paradigm. Marmoset monkeys with unilateral lesions of either antOFC (area 11) or vlPFC (area 12) were scanned immediately following exposures to a fearful (rubber snake and darkness) or ‘safe’ context, and the differences in regional ligand uptake were compared. Analysis of three antOFC lesioned animals so far has revealed that, in the intact hemisphere, FDG uptake in the amygdala was significantly increased in response to the fearful context compared to the ‘safe’ context. However, this difference in FDG uptake between the two contexts was not seen in the amygdala of the lesioned hemisphere, being high in both fear and ‘safe’ contexts. These results will be compared with those of the vlPFC, providing important new insight into the top-down control of the subcortical emotion network. It may also progress our understanding of the neurocognitive mechanisms underlying marked symptomatic differences in mood and anxiety disorders, which in turn could lead to the refinement of diagnoses and the individual tailoring of therapeutic strategies.


Poster

531. Decision Making and Attention: Prefrontal Cortex

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM
Astrocytes are involved in cognitive flexibility

Authors: *A. T. BROCKETT, E. A. LAMARCA, B. A. BRIONES, E. GOULD;
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Abstract: Astrocytes are a relatively understudied cell type in the brain despite being numerous and structurally complex. Across evolution, animals with more complex brains capable of more complex behavior also have more numerous and morphologically complex astrocytes (Oberheim et al. 2008). This suggests that astrocytes may be active participants in complex cognition. Recent evidence suggests that running increases astrocyte size and endfeet coverage as well as dendritic complexity in the medial prefrontal cortex (mPFC) and hippocampus. These running-induced changes in both astrocytes and neurons were associated with enhanced cognitive functioning (Brockett et al., 2015). Astrocytes are capable of regulating synaptic plasticity through the release of small molecules such as D-serine (Henneberger et al., 2010); moreover, the collective action of astrocyte Ca²⁺ signaling has been shown to regulate gamma oscillations in the hippocampus, which can influence object recognition memory (Lee et al., 2014). The mPFC is highly involved in the maintenance of cognitive flexibility, but what a reduction in the number of astrocytes in the mPFC does to such behavior and the surrounding neurons remains relatively unknown. As a first step toward addressing these questions, we reduced the number of astrocytes in the mPFC using a low dose of the astrocyte-specific toxin L-alpha-aminoadipic acid (L-AAA) (Banasr & Duman, 2008) in adult male Sprague Dawley rats before testing them on the attentional set-shifting task (ASST). Astrocyte reduction in the mPFC resulted in impairment on the mPFC-dependent portion of the ASST compared to vehicle-treated controls. L-AAA did not result in any changes in neuron density, neuronal cell body size, dendritic spine density, dendritic spine size or neuronal expression of immediate early genes in the mPFC, suggesting that astrocyte reduction occurred without noticeable negative consequences to surrounding neurons. Future studies will use more directed strategies for disrupting astrocyte signaling to determine the influence of astrocytes on cognitive flexibility.

Disclosures: A.T. Brockett: None. E.A. LaMarca: None. B.A. Briones: None. E. Gould: None.

Poster

531. Decision Making and Attention: Prefrontal Cortex

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM
Program#/Poster#: 531.03/Z7

Topic: F.02. Animal Cognition and Behavior

Support: BBRF NARSAD 23017

Title: Prefrontal cortico-thalamic network connectivity for cognitive control

Authors: *J. M. PHILLIPS¹, N. A. KAMBI¹, S. R. KECSKEMETI², Y. B. SAALMANN¹;
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Abstract: The prefrontal cortex (PFC) has an established role in cognitive control, that is, flexibly adapting behavior according to goals, context and rules. Anatomical tracer studies indicate that the lateral PFC has dense, reciprocal connections with the parvocellular compartment of the medio-dorsal thalamic nucleus (MD) (Ray & Price, 1993 J Comp Neurol 337:1). However, the contribution of MD to cognitive control remains relatively unexplored. We hypothesize that PFC and MD interact to support cognitive control, specifically that MD regulates information processing across PFC. In this framework, MD may selectively activate different groups of PFC neurons based on cognitive control demands. To enable our investigation of MD-PFC connectivity, we performed high resolution (1.0 mm isotropic) diffusion weighted imaging on 4 anesthetized macaque monkeys using the GE MR750 3T scanner with a 16-channel receive-only head coil (MRI Instruments). We acquired 60 diffusion directions (b=1000 s/mm² and NEX=14) and 112 b=0 images. We used FSL for EPI distortion, eddy current and motion correction, prior to Bayesian estimation of diffusion parameters and probabilistic tractography. Our tractography results were broadly consistent with anatomical tracer studies. However, diffusion tractography had the advantage of delineating projection zones within areas specific to each monkey, which cannot be precisely ascribed on the basis of published tracer data. Based on probabilistic cortical connectivity patterns, we subdivided MD into three subdivisions: medial, dorsal and lateral subdivisions predominantly connected with orbitofrontal cortex, medial PFC and lateral PFC, respectively. The lateral subdivision occupied the relatively largest portion of MD and seemingly corresponded to parvocellular MD. Importantly, circumscribed zones of the lateral MD subdivision connected with both PFC areas 46 and 9/46. This suggests that there is an indirect cortico-thalamo-cortical pathway between areas 46 and 9/46 via MD, in addition to the direct cortico-cortical pathway between these PFC areas. Our data implies that the parvocellular MD is well positioned to coordinate information transmission between lateral PFC areas. The diffusion tractography data also allow us to target MRI-compatible linear microelectrode arrays to interconnected sites between the areas 46, 9/46 and parvocellular MD, for simultaneous multi-site recordings of thalamo-cortical dynamics during cognitive control. We propose that a general function of MD, and other higher-order thalamic areas like the pulvinar, is to regulate cortical processing according to behavioral demands.

Poster
531. Decision Making and Attention: Prefrontal Cortex

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#/Poster#: 531.04/Z8

Topic: F.02. Animal Cognition and Behavior

Support: NIH R01 DA037229

Title: A bayesian method of categorizing neurons based on functional properties

Authors: *T. BLANCHARD, S. T. PIANTADOSI, B. Y. HAYDEN;
Brain and Cognitive Sci., Univ. of Rochester, Rochester, NY

Abstract: Historically, it has often been useful to classify neurons as belonging to one of a few discrete functional categories based on the variables they are sensitive to. However, there has been a recent surge in research finding that cortical neurons often have mixed selectivities and lack discrete functional categories. Are neurons best thought of as having a random mix of tuning to variables or as belonging to discrete categories? To investigate this, we developed a Bayesian clustering method of classifying the tuning properties of neurons. This method allows us to test for whether the tuning properties of neurons in a dataset are best characterized as coming from the same distribution or from multiple distributions (categories). Our approach also allows us to determine when the data leaves high uncertainty about the existence of clusters. It also allows us to test for systematic relationships between how neurons encode one variable and another variable. We demonstrate these methods on data from the prefrontal cortex.


Poster
531. Decision Making and Attention: Prefrontal Cortex

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#/Poster#: 531.05/Z9
**Abstract:** Cortical activity correlates of binary decision making have been well-described in nonhuman primates (e.g. Roitman, J.D. & Shadlen, M.N., 2002) and more recently in rodents (Jahans-Price et al. 2014; Raposo et al. 2014; Hanks et al. 2015). In both cases cortical neurons display ramping activity, suggesting they could be integrating evidence for choice alternatives. Meanwhile, the rodent hippocampus is implicated in decision making and in planning future actions. For example, during moments of quiescence in decision making tasks, decoded hippocampal spatial representations appear predictive of future behaviour (Foster and Pfeiffer, 2013; Johnson and Redish, 2007). During this phenomenon, the animal’s head often orients towards and switches between potential options, as rats appear to consider the alternatives in a process described as vicarious trial and error (VTE). In order to investigate whether hippocampal and prefrontal information processing is coordinated during VTE, we recorded local field potential (LFP) and multiple single neuron action potentials from medial PFC units and hippocampal LFP from five adult rats during a spatial decision making task. PFC units were recorded from three rats using an array of independently moveable tetrodes and from two rats using multi-shank silicon probes. The task, based on Powell and Redish (2014), requires a rat to run left or right at a T-junction in order to gain a reward - one side of the maze is rewarded in each session. The side rewarded is chosen pseudorandomly and in a subset of sessions is switched mid-session. Rule variation is used to discourage automated behaviour and increases instances of VTE. During moments when rats appear to be considering route options at T-junctions, we analyse information processing in the prefrontal cortex, oscillations in the hippocampus and the interactions between both structures. Of the prefrontal units recorded we found a subset of 41% which displayed side-selectivity, firing at higher rates on either the left or right side of the maze. Within VTE events prefrontal neurons selective for a particular maze side displayed higher activity if the rat subsequently entered that side, compared to entering the alternative side. We also saw an increase in theta power during VTE compared to equivalent slow movement. These results demonstrate that PFC represents future outcomes and participates with the hippocampus during the planning that occurs in VTE events.

**Disclosures:** T. Jahans-Price: None. R. Bogacz: None. M.W. Jones: None.
Title: Synchronous beta oscillations in the fronto-striatal loop for behavioral rule switching in non-human primates

Authors: *F. GERARD-MERCIER, K. TANAKA; RIKEN BSI, Cognitive Brain Mapping Lab., Saitama, Japan

Abstract: Behavioral flexibility is crucial for survival in a versatile environment. Behavioral rules (sets of stimulus-action contingencies given a certain environmental setting) have to be constantly evaluated for current relevance, and switches between rules have to occur whenever the environmental setting changes. Crucially, in everyday life, which rule to follow in which setting is implicit and uncued, unlike in most experimental studies of behavioral flexibility. We therefore sought to investigate in non-human primates the neural correlates of uncued behavioral rule switching. We trained monkeys to perform a task analog to the Wisconsin Card Sorting Test, in which they had to perform different sets of actions in response to the same stimuli depending on the frequently changing current rule. On each trial, a colored shape was presented (sample), then, three test items appeared around the sample, one matching the sample in shape (but not in color), a second in color (but not in shape), and the last in neither shape nor color. Monkeys had to touch the test item that matched the sample in shape, or in color, depending on the current rule. Importantly, which rule (shape or color) had to be followed was not cued, and the sample-action contingencies were fixed, so that errors reflected failure in rule maintenance. The rule switched whenever the monkey had made 6 consecutive correct responses, after a minimum of 12 consecutive trials. While the monkeys performed this task, we carried out simultaneous recordings in three sites of the fronto-striatal loop crucial for executive function: the dorsal bank of anterior cingulate sulcus (part of ACC), dorsolateral PFC (area 46d, within the principal sulcus), and the dorsal part of anterior caudate head, the part of the striatum known to receive the most extensive inputs from ACC and dLPFC. Preliminary analyses showed prominent low beta (~15Hz) LFP oscillations in all three sites both during fixation (just before sample onset) and around feedback. Coherence measures indicated that these oscillations were synchronized across all three sites. Moreover, both power and coherence were modulated by rule (both during fixation and around feedback), and whether the response was correct or wrong (around feedback). These data, consistent with previous reports, support the idea that low beta oscillations provide communication channels for the evaluation and maintenance of the current...
behavioral rule, and that dynamical changes in subpopulations of synchronized neurons provide the substrate for behavioral flexibility.

**Disclosures:** F. Gerard-Mercier: None. K. Tanaka: None.

**Poster**

531. Decision Making and Attention: Prefrontal Cortex

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#: 531.07/Z11

**Topic:** F.02. Animal Cognition and Behavior

**Title:** Check or Go? Anxiety based checking behavior in rhesus monkey

**Authors:** *M. BOSC*¹², B. BIOULAC¹², N. LANGBOUR³, T.-H. NGUYEN¹², M. GOILLANDEAU¹², B. DEHAY¹², P. BURBAUD¹²⁴, T. MICHELET¹²;
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**Abstract:** Checking compulsions is among the most common behavioral features of obsessive-compulsive disorder (OCD), an anxiety disorders reaching 2-3% of the population. Checking compulsion derives and escalates from once-functional behavior. It is thought to be a behavioral attempt to relieve anxiety caused by high levels of doubt and uncertainty. Numerous studies suggest that alteration of prefrontal cortices would lead to abnormal cognitive control of action (e.g. conflict monitoring or error detection) and could be related to compulsive checking behavior. However the physiology of checking behavior remains poorly understood and no concrete and reliable model of physiological checking has been developed so far. The present behavioral study tends to characterize physiological doubt and checking behavior in non-human primates (NHP). To do so, we designed a novel behavioral task to study electrophysiological correlates of decision making, doubt and checking in NHP. We trained two rhesus monkeys (Macaca mulatta) on the Check-or-Go task while recording their frontal EEG activity. We also collected saliva samples to quantify cortisol concentration along sessions in order to correlate this biological marker of anxiety with checking behavior. Our behavioral paradigm allows the animal to multiple-check and potentially change the availability of the reward before taking the final decision leading to that reward. By manipulating the ambiguity of the visual cue embedding the reward status (reward operational cue), we successfully modulated animal uncertainty and created doubt. Behavioral results showed that the animal uncertainty level influenced not only
performances and reaction times but also checking behavior rate. Fronto-central EEG potentials were also modulated by visual cues’ ambiguity level and checking decision. Daily cortisol quantification revealed a positive correlation between monkeys’ checking behavior and anxiety level. Taken together, our study demonstrated that the Check-or-Go task is a valid behavioral tool to study the impact of ambiguity on doubt and the subsequent adaptive checking behavior and will thereby help us provide new insights into OCD mechanisms.


Poster

531. Decision Making and Attention: Prefrontal Cortex

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 531.08/Z12

Topic: F.02. Animal Cognition and Behavior

Support: Swedish Brain foundation

Title: The role of prefrontal fast-spiking parvalbumin interneurons in attention

Authors: *S. K. ÄHRLUND-RICHTER, H. KIM, M. CARLÉN; Karolinska Institutet, Stockholm, Sweden

Abstract: Attention allows us to filter out irrelevant sensory information in favor of relevant information. The signatures of attention have been well described in visual cortex but the neural sources and computation responsible for the control of attention has not been established. The medial prefrontal cortex (PFC) oversees a large range of higher cognitive functions and has been demonstrated as central in the control of attention. PFC influence visual processing but the cellular and physiological underpinnings have not been characterized, including the contribution of the local PFC network. Cortical fast-spiking parvalbumin interneurons (FS-PV) are known for their involvement in coordination of the local circuitry and generation of gamma oscillations. More recently, prefrontal FS-PV interneurons have been shown to be recruited by behavior and to even play a direct functional role in expression of behavior. To investigate the role of mPFC FS-PV neurons in the control of attention we conducted extracellular single-unit recordings in PV-Cre mice performing an attention task, the 3-choice serial reaction time task (3-CSRTT). During the task animals are required to allocate attention in order to detect a brief visual stimulus (cue) in an operant chamber, and to report the cue location by nose poking. We analyzed the population activity of FS-PV neurons and WS putative pyramidal neurons during attentional
processing and found that the activity FS-PV neurons but not WS neurons predict successful attentional processing. In addition, successful attentional processing was correlated to an increased level of gamma oscillation in mPFC. Using optogenetics we could demonstrate a direct functional role of mPFC FS-PV neurons and gamma oscillation in the control of attention. Moreover, optogenetic manipulation of mPFC FS-PV neurons enables enhancement of attentional processing.


Poster

531. Decision Making and Attention: Prefrontal Cortex

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

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Title: Methylphenidate reduces noise correlations and improves neural ensemble coding in the primate lateral prefrontal cortex

Authors: *S. Tremblay¹, F. Pieper², A. Sachs³, J. Martinez-Trujillo⁴; ¹Integrated Program in Neurosci., McGill Univ., Montreal, QC, Canada; ²Univ. Med. Ctr. Hamburg-Eppendorf, Hamburg, Germany; ³Ottawa Hosp. Res. Inst., Ottawa, ON, Canada; ⁴Western Univ., London, ON, Canada

Abstract: Methylphenidate (MPH), commonly known as Ritalin©, is one of the most widely prescribed drugs to treat patients with attention deficit hyperactivity disorder (ADHD). Despite its proven effectiveness in both children and adults, we currently have a limited understanding of the neural mechanisms by which the drug improves cognitive performance in both ADHD patients and normal controls. Using multielectrode arrays implanted in the primate prefrontal cortex, we show that MPH, at low doses that improve cognition, reduces noise correlations within prefrontal neuronal ensembles without substantially modulating the overall level of neuronal activity. This effect improved the neural ensembles performance at encoding the focus of attention during a behavioral task. Our results identify a novel network-level mechanism of action of MPH on information coding by prefrontal neuronal ensembles in the primate brain.
**Disclosures:** S. Tremblay: None. F. Pieper: None. A. Sachs: None. J. Martinez-Trujillo: None.

**Poster**

**531. Decision Making and Attention: Prefrontal Cortex**

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**Title:** Neural mechanisms of shape discrimination under partial occlusion: a circuit model of V4 and prefrontal cortex

**Authors:** *H. CHOI¹², A. FYALL², E. SHEA-BROWN¹, A. PASUPATHY²;
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**Abstract:** The primate visual system has an exquisite ability to recognize objects in natural scenes even though most objects appear partially occluded. The robustness of shape discrimination in the brain has yet to be matched in computer vision. How the brain recognizes partially occluded shapes, therefore, is an important question. Here we investigate possible underlying neural mechanisms using a computational model of V4 and prefrontal cortex (PFC) based on our recent electrophysiological recordings from the macaque. We propose that feedback signals from PFC to V4 contribute to the representation and discrimination of partially occluded shapes. We studied responses of V4 and PFC neurons while monkeys discriminated pairs of shapes under varying degrees of occlusion. In V4, neurons showed weakened shape selectivity early in their responses as the occlusion increased. Later in time, around 200 ms after the stimulus onset, the responses peaked again, and the amplification of selectivity was more prominent for the stimulus under intermediate level of occlusion. Interestingly, neurons in PFC, which receive signals from V4, responded strongly to occluded stimuli but very weakly to
unoccluded stimuli. Moreover, PFC responses peaked in between the early and the later peaks of V4 responses. These observations are consistent with the hypothesis that PFC provides feedback modulation responsible for the later increase in shape selectivity in V4 under occlusion. Based on these experiments, we constructed a model composed of two layers of neurons corresponding to V4 and PFC. Our model includes two populations of V4 neurons, which respond preferentially to two different test stimuli. Each V4 population gives excitatory feedforward inputs to two different populations of PFC neurons, whose responses are also modulated by an occlusion-dependent gain function. The PFC output is fed back to the V4 neurons with the matched shape preference. The model outcome agrees with the experiments, predicting low V4 shape selectivity under occlusion during the earlier peak, which increases later during the second peak. In addition, our preliminary data indicates that a gain function that merely detects the presence of occlusion promotes shape selectivity as well as a gain that gradually increases as degree of occlusion increases. Our combined modeling and electrophysiology are consistent with the idea that feedback from PFC to V4 plays a significant role in maintaining shape discrimination under partial occlusion. These results suggest a possible role of feedback for object recognition in the ventral visual pathway.


Poster

531. Decision Making and Attention: Prefrontal Cortex

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MEDI

Title: Theta-phase reset and interareal burst synchronization to gamma activity co-occur in a theta-gamma coupled attention network

Authors: *B. VOLOH, T. WOMELSDORF;
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Abstract: Selective visual attention relies on mechanisms that integrate goal-relevant events through a distributed network of neurons encoding goal-relevant information. Yet it is unclear
how external events are converted into an endogenous signal driving network formation. Ongoing but unrelated rhythmic activity fluctuations across cortical sites may be phase reset by a relevant external event, thus imposing a common neural context onto which goal-relevant information can be mapped [1]. Alternatively, transient burst firing modulate the effective connectivity of network nodes, whereby the strong post-synaptic activation may drive relevant neurons to an active processing mode [2]. Importantly, both phenomena may not be mutually exclusive: bursts may provide the drive for widespread phase resetting, or a phase reset may bring neurons into burst firing modes. Thus, phase resets and bursts could underlie the updating of goal relevant representation in larger-scale networks. To test for these possible interactions of burst firing and phase reset, we have analyzed the local field potential (LFP) and neuronal burst firing in anterior cingulate and prefrontal cortices (ACC/PFC) in macaques performing a selective attention task. Shifting attention depended on the integration of stimulus feature information with cue information. In previous work, we have shown that during the attentional stimulus selection, neuronal spike bursts in ACC/PFC synchronize to gamma and beta LFP oscillations [2]. At the same time, LFPs in the ACC phase coupled low frequency 5-10Hz theta phases with gamma activity in lateral PFC [3]. Here, we characterize these phenomena and their putative relation. First, we found that theta phase-providing LFPs showed a phase reset during correct but not erroneous attention shifts. Second, these LFPs hosted single-neuron bursts that were coupled to distant gamma in direct relation to the strength of theta-gamma coupling. Finally, burst-LFP synchronization showed the opposite relation with network strength at beta frequencies, illustrating a segregation of theta-gamma interactions from processes in the beta-band. These results show that the recruitment of behaviorally relevant neural ensembles occurs in the presence of both theta-phase resetting and bursts synchronizing with distal gamma. Both phenomena occur in the same cortical site, and are directly correlated with the degree of ensuing theta-gamma coupling, suggesting that they contribute to the formation of goal-relevant networks. [1] van Atteveldt, N. et al (2014). Neuron. 81:1240-1253 [2] Womelsdorf, T. et al (2014) Curr. Biol. 24:1-9 [3] Voloh, B. et al (2014). Poster, SFN 2014

Disclosures: B. Voloh: None. T. Womelsdorf: None.

Poster

531. Decision Making and Attention: Prefrontal Cortex

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Title: Opposite development of short- and long-range anterior cingulate pathways in autism

Authors: *B. ZIKOPOULOS, Y. J. JOHN, J. TEPE, H. BARBAS;
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Abstract: Autism spectrum disorders (ASD) have been consistently linked with changes in brain connectivity that disrupt neural communication, especially in frontal networks. Our prior work indicated that compared to neurotypical controls, the white matter below anterior cingulate cortex (ACC) area 32 (A32) of adults with ASD has an elevated proportion of thin axons that likely connect ACC with nearby areas, and a reduced proportion of thick axons that likely extend over long distances (Zikopoulos and Barbas, Journal of Neuroscience, 2010; Zikopoulos and Barbas, Frontiers in Human Neuroscience, 2013). These findings are in agreement with reported gross changes in the volume and structural integrity of frontal white matter in ASD. It is not clear however, whether changes in the fine structure of axons below ACC and the pathways they form appear early or late in development. To address this question we used post-mortem brains of children (ages 3-10 years) and adults (ages 30-44 years) with and without ASD, and systematically sampled and imaged the white matter below ACC at very high resolution in the electron microscope. We used unbiased methods to quantitatively study the density and ultrastructure of individual myelinated axons. In the ASD group the mean inner and outer diameters of myelinated axons showed a decrease from childhood to adulthood, whereas in the control group they showed an increase. Further analysis of thin and thick axon populations that form short-range cortical and long-range pathways showed significantly different developmental trajectories. The proportion of thin axons present in childhood and adulthood remained roughly constant in neurotypical controls, whereas it increased in individuals with ASD. In contrast, the relative proportion of thick axons in young and adult individuals increased from childhood to adulthood in the control group, but remained flat in ASD. These findings are consistent with the idea that changes in axonal architecture affect in distinct ways short- and long-range cortical pathways, modifying the interactions of nearby or distant brain structures. Axon diameter affects conduction velocity, which in turn influences physiological processes including neural synchrony and communication, in processes that are disrupted in ASD. Moreover, our preliminary data suggest that changes in the relative size composition of axons in the white matter below ACC appear later in life and may reflect continued disturbances in axon growth and guidance in autism.

The structure of the cerebral cortex is generally viewed in two ways: as a mosaic of indistinguishable six-layered columnar circuits, and as a patchwork of discrete architectonic areas. These seemingly distinct views converge on the idea that the basic six-layer structure is identical across the cortex, and that all areas perform similar canonical functions, differing only by their connection patterns. But a growing body of evidence suggests that the organization of the cortex is best understood by systematic variation. The cortex is neither a uniform six-layered structure, nor a collection of dissimilar areas with sharp boundaries -- it is best characterized as a spectrum, in which structural features change smoothly, and discontinuities are rare. To investigate the nature of the cortical spectrum, we studied key structural properties of different cortical areas, representing all systems, including the densities of neurons, glia and myelinated axons, ranging from the pial surface to the underlying white matter tracts. These data allowed us to divide a large number of areas into a much smaller number of robust types of cortices that differ in systematic ways. Overall, laminar density of neurons and myelin were informative and changed gradually from low to high levels in limbic, association, and the most specialized primary cortices. Laminar-specific changes in cell morphology, especially in layer 3, were also useful in discriminating and clustering areas. Categorization of many different cortical areas into a few cortical types within each system allowed us to identify analogies between different systems and processing streams. Our analysis also highlights specializations in cortical structure that reflect specialized processing streams and functions. Rather than being taxonomical, this cortical topology serves as a powerful framework within which to understand brain structure and function. These broad cortical types provide useful insight into patterns of cortico-cortical connectivity, which are often altered in neurologic and psychiatric disorders. The pattern of
connections between two given cortical areas is not simply a function of inter-areal distance, but
depends quantitatively and qualitatively on the types to which the areas belong. The cortical
spectrum perspective allows for a principled approach to determine functional cortical
hierarchies, and leads to testable hypotheses regarding neural development and the evolution of
the mammalian cortex.

Disclosures:  Y.J. John: None. B. Zikopoulos: None. M. Garcia Cabezas: None. H. Barbas:
None.

Poster

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Support: JSPS KAKENHI 25890024

Title: Fine timescale dopaminergic modulation of prefrontal neuronal circuit activity

Authors: *K. TAO, S. FUJISAWA;
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Abstract: The prefrontal cortex is one of the major projecting targets of dopamine neurons in the
ventral tegmental area (VTA). These mesoprefrontal dopamine neurons has been considered to
modulate prefrontal network activity tonically through volume transmission as well as phasically
in a synaptic fashion. However, the effect of phasic firing of dopamine neurons on prefrontal
circuit activity in a sub-second timescale remains elusive. Here we show that, in a classical
reward conditioning paradigm under head-restrained apparatus using mice, fast-spiking
interneurons (FS INs) in the medial prefrontal cortex (mPFC) respond both to water rewards and
reward predicting cues. Both water rewards and optogenetic stimulation of dopaminergic
neurons in the VTA elicited firing of FS INs in the mPFC. Conversely, the vast majority of
regular-spiking neurons were inhibited by these manipulations. Subcutaneous injection of
SCH23390, a dopamine receptor D1 (D1R) antagonist, suppressed firing of FS INs induced by
rewards. These results suggest that mesoprefrontal dopaminergic neuronal firing inhibit overall
neuronal activity in the mPFC in a D1R-dependent manner, presumably in concert with
 glutamatergic inputs from lower order cortices or thalamic nuclei.

Disclosures:  K. Tao: None. S. Fujisawa: None.
Poster

531. Decision Making and Attention: Prefrontal Cortex

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Topic: F.02. Animal Cognition and Behavior

Title: Inter-areal spiketrain correlations of anterior cingulate and prefrontal cortex during attention shifts: Cell-type, anatomical, and temporal specificity

Authors: *M. OEMISCH¹, S. WESTENDORFF¹, S. EVERLING², T. WOMELSDORF¹;
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Abstract: Anterior cingulate cortex (ACC) and dorsal prefrontal cortex (PFC) are believed to coordinate activity during goal-directed behavior to identify, select and monitor relevant sensory information. The ACC is suggested to monitor and bias attentional selection, while lateral and dorsal prefrontal areas are involved in the actual implementation of attentional control. To this point it is unclear how the distributed brain areas interact to integrate their respective attention information, and studies investigating direct interactions between these areas have been sparse. Here we recorded >2000 pairs of cells and tested whether spiketrain interactions of neurons across macaque ACC and PFC reflect such coordination during stimulus selection in a spatial attention task. For this task monkeys had to attend one of two colored stimulus gratings and discriminate a transient clockwise or counter-clockwise rotation in order to receive liquid reward. The attentional target was indicated by the color of a fixation point. Spiketrain correlations were computed using joint peri-stimulus time histograms (JPSTHs) and confirmed with Pearson correlations. We found that spiketrain correlations emerged shortly after an attention cue and were evident for 50-200ms time windows. Increased firing correlations were strongest for neuron pairs in area 24 (ACC) and areas 8 and 9 (dorsal PFC), they were independent of overall firing rate modulations, and firing events in PFC preceded firing events in ACC. For a subset of cell pairs from ACC and PFC the observed functional spiketrain connectivity carried information about the direction of the attention shift, whereby neurons encoding contralateral attention shifts engaged in increased synchronization, while neurons encoding ipsilateral shifts engaged in decreased synchronization. Reliable firing correlations were evident across area boundaries for neurons with broad spike waveforms (putative excitatory neurons) as well as for pairs of putative excitatory neurons and neurons with narrow spike waveform (putative interneurons). These findings reveal that stimulus selection is accompanied by slow time scale firing correlations across those ACC and PFC subfields implicated to control
and monitor attention. This functional coupling could index the selective routing of goal-relevant information and may reflect the transient coordination of larger, reciprocally interacting brain networks at a characteristic 50-200ms time scale.

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**Poster**

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**Title:** The representation of abstract information that guides decision making in PFC of rats

**Authors:** *S. TERADA*¹², Y. SAKURAI¹, H. NAKAHARA², S. FUJISAWA¹; ¹Kyoto Univ., Saitama, Japan; ²RIKEN BSI, Wako, Japan

**Abstract:** Decision making is often guided by prediction of outcome such as reward. Both reward prediction and action selection need to be acquired and generated on neuronal representation that reflects sensory inputs or information of the environment. Critically, the representation should not be merely a collection of sensory inputs but instead, should be organized to encode and abstract information required for prediction and selection. So far mostly using a simple sensory cue, the prediction and selection have been studied in the field of value-based decision making, however, surprisingly little is known about the neural representations for the abstraction of the required information during the acquisition and generation. For this purpose, we have newly developed a behavioral task for rats, cue-combination task. Central for this task is that the correct choice of action should be determined by a combination of two cues (sound and odor cues); they cannot be determined by either of cues alone. In each session, sound and odor cues will be presented simultaneously for a couple of seconds to the rat, followed by a choice period, i.e., right or left lever to pull for getting a reward. Large-scale extracellular single
unit recordings in the prefrontal cortex (PFC) during this task revealed that several neurons, referred to here as combination cells, discharge at the time of presentation of only one of the combination patterns of the sound and odor cues. Most of the rest populations of PFC neurons were activated in a choice-dependent (i.e., left or right) manner. We hypothesize that PFC have the different function for this task, and their interaction might form and represent abstract information to solve exacting decision problems.

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Poster

531. Decision Making and Attention: Prefrontal Cortex

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Title: Markers of plasticity suggest higher vulnerability in prefrontal limbic cortices in primates

Authors: *M. GARCIA-CABEZAS, H. BARBAS;
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Abstract: Limbic cortices are at the base of every cortical system and show particular architectonic features, such as absence of layer IV or a poorly developed layer IV, lower neuron density and lower myelin content. They have widespread connections with sensory and other high-order association areas and strong reciprocal connections with the amygdala and the hypothalamus. In contrast, eulaminate cortices have six layers, higher neuron density and higher myelin content than limbic cortices. Eulaminate areas have more restricted cortical connections and receive fewer projections from the amygdala and the hypothalamus, which are not reciprocal. At the cellular level, pyramidal neurons in limbic areas have more branched and more spinous dendritic trees than the eulaminate areas. These architectonic, connectional and cellular features suggest that the limbic cortices are generalists in cortical processing while eulaminate cortices are specialists. These features also suggest that limbic cortices are more plastic than eulaminate cortices. To further investigate features that differentiate limbic and eulaminate areas we studied the distribution of CamKII and the glutamate receptor subunit NR2B, two markers of
synaptic plasticity, and of GFAP, a marker of astrocyte activation, in anterior cingulate (limbic) and dorsolateral prefrontal (eulaminate) areas in rhesus monkeys. Anterior cingulate areas 25 and 32 have higher density of CamKII and NR2B in all their layers. In dorsolateral eulaminate areas 9 and 46 the middle layers show lower expression of these markers. GFAP is denser throughout all layers of limbic cortices, while in eulaminate areas its expression is mostly restricted to layers I-IIIa and VI. Limbic cortices are implicated more frequently in neurological and psychiatric diseases than eulaminate cortices. For instance, in Alzheimer’s disease neurons in limbic cortices have more tau inclusions than eulaminate areas and in depression the subgenual cingulate cortex is critically affected. These findings suggest that limbic cortices are more plastic but also may be more vulnerable to insults and disease than eulaminate cortices.

Disclosures: M. Garcia-Cabezas: None. H. Barbas: None.

Poster

531. Decision Making and Attention: Prefrontal Cortex

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Title: Effects of focal prefrontal ischemic lesions on delay discounting and ultrasonic vocalizations in the rat

Authors: *R. A. DÉZIEL, R. A. TASKER;
Univ. of Prince Edward Island, Charlottetown, PE, Canada

Abstract: Stroke is the most common cause of long-term disability in adults worldwide, and it has been estimated that approximately 30% of stroke survivors experience cognitive impairments persisting for at least three months post-stroke. Cognitive deficits post-stroke are characterized by various symptoms including aphasia, attentional deficits, inhibitory control deficits, and social/emotional dysfunction. Many of these cognitive deficits occur because of damage to the prefrontal cortex (PFC), which has been heavily implicated as the seat of higher order cognitive functions in the brain. An improved understanding of the role of the PFC in cognitive functions post stroke requires appropriate preclinical models. Utilizing bilateral microinjections (400
pmol) of the vasoconstricting peptide endothelin-1 (ET-1) into either the medial or orbital PFC in male SD rats (n = 10-12/group) we produced localized ischemic lesions in these areas of the PFC (or sham). The effects of these lesions on cognition were assessed using tests measuring inhibitory control (delay discounting) as well as by recording and analyzing ultrasonic vocalizations produced during behavioural testing both pre- and post-stroke. Post-mortem lesion size and location was determined histologically. The combination of localized damage and behavioural tests of cognition shows potential for developing a model of post-ischemic cognitive dysfunction that will support subsequent studies of cognitive rehabilitation and cortical neuroplasticity.

Disclosures: R.A. Déziel: None. R.A. Tasker: None.

Poster

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Title: Downregulation of mediodorsal thalamic activity reduces GABAergic neurotransmission in the medial prefrontal cortex, disrupting the E/I balance, and impairing cognitive function

Authors: *B. R. FERGUSON¹, W. J. GAO²;
¹Neurobio. and Anat., ²Drexel Univ. Col. of Med., Philadelphia, PA

Abstract: The mediodorsal (MD) thalamus plays a critical role in cognition through its extensive innervation of the prefrontal cortex (PFC). The MD's dense glutamatergic afferents synapse directly onto excitatory pyramidal neurons, and inhibitory GABAergic interneurons in the medial PFC (mPFC). GABAergic interneurons are essential in coordinating the activity of neighboring neurons and enhancing information processing within their local circuitry. Given the mPFC’s classic implication in cognition and executive function, gaining a richer understanding of the subcortical structure driving GABAergic tone in this region is of fundamental importance. We hypothesize that the MD provides preferential synaptic drive to prefrontal inhibitory neurons, aiding in the regulation of local circuit activity and optimizing behavioral outcomes. To explore this we utilized the pharmacogenetic strategy, DREADDs, to downregulate MD activity and evaluate the consequences for working memory and cognitive flexibility, as well as the underlying alterations in mPFC synaptic transmission. Following MD inhibition, we observed
impairments in both working memory and cognitive flexibility as demonstrated by poorer performance in the T-maze and Cross-maze set-shifting task respectively. Additionally, we see reductions in mPFC GABAergic activity concomitant with elevations in excitatory neurotransmission. This suggests that the MD is crucial in maintaining the E/I balance in the mPFC circuitry, and the resultant cacophony generated when MD afferent drive is decreased may underlie behavioral deficits. Ongoing studies involve seeking to rescue these deficits by restoring GABAergic activity with a GABAA-positive allosteric modulator, indiplon.

Disclosures: B.R. Ferguson: None. W. Gao: None.

Poster

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Title: Representational transition from behavioral tactics into action by neurons in primate posterior medial prefrontal cortex

Authors: *Y. MATSUZAKA, A. SASAGAWA, H. MUSHIAKE; Tohoku Univ., Sendai, Japan

Abstract: Use of various response tactics (i.e. the protocol to decide action) is an important aspect of adaptive behavior. Previous physiological studies revealed that the posterior medial prefrontal cortex (pmPFC) of primates participates in the selection of voluntary action only when the task required the selection of valid tactics as well. Because the selection of tactics and the selection of action are different levels of decision making, we studied how this area contributes to these processes. Two monkeys were trained to perform a two-choice arm reaching task in which the monkeys chose one of the multiple tactics to select the direction of reaching movements. The selection of the tactics and the selection of the reaching direction were temporally separated. In experiment 1, a color cue directly instructed the tactics (either reach to or away from the subsequent spatial cue) of the forthcoming reaching movement. After a variable length of delay period, a spatial cue (either left or right) appeared, prompting the monkeys to reach either ipsilaterally or contralaterally to its location, depending on the tactics instructed by the preceding color cue. Neurons in the pmPFC exhibited selective activity for the cued tactics during the delay period, but such activity quickly disappeared and was replaced with
action-selective activity once the action was determined. In experiment 2, the monkeys were trained to search the valid tactics using a series of color cues. After the tactics was determined, an additional color cue instructed the monkeys to select the action. We found that numbers of pmPFC neurons which encoded the tactics during its search or selection also encoded the forthcoming action after the target of arm reaching was determined. Further, the appearance of action selective neuronal activity was frequently accompanied by the reduced selectivity for the tactics. The switch of neuronal representation of task-relevant information indicates that the pmPFC contributes to the transformation of the appropriate tactic into action.

Disclosures: Y. Matsuzaka: None. A. Sasagawa: None. H. Mushiake: None.

Poster

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Title: Effect of amygdala lesions on local field potentials in the primate prefrontal cortex during a reward-guided task

Authors: *C. P. MOSHER¹, S. TAMANG¹, E. A. MURRAY², P. H. RUDEBECK¹; ¹Friedman Brain Inst. and Dept. of Neurosci., Ichan Sch. of Med. at Mount Sinai, New York City, NY; ²Lab. of Neuropsychology, Natl. Inst. of Mental Hlth., Bethesda, MD

Abstract: Reward-guided behaviors require functional interactions between the amygdala and the prefrontal cortex, specifically the orbital and medial divisions (OFC and MFC, respectively). In monkeys, bilateral excitotoxic lesions of the amygdala attenuate reward-value signals of individual neurons recorded from the OFC, but not the MFC (Rudebeck et al., 2013, Neuron, 80: 1519). The response properties of single neurons, however, only reflect the local processing and output of an area; a more complete understanding of this network might be informed by considering population-level activity and the inputs to an area, which can be studied using local field potentials (LFPs). To better determine the population dynamics of reward-value coding in the amygdala-MFC-OFC network, we performed time-frequency analysis on LFPs recorded from the OFC and MFC of three monkeys engaged in a stimulus-choice task. Recordings were
made both before and after excitotoxic lesions of the amygdala. On each trial of the task, monkeys were free to choose between two sequentially presented visual stimuli that were each associated with different amounts of fluid reward. Because the LFP is thought to represent the population-level input to a structure, we expected to find signatures in the prefrontal LFP that (1) reflect the reward-value associated with a visual stimulus, (2) are altered when value signals from the amygdala are removed, and (3) precede or coincide with local single cell activity. Our analyses suggest that the visual stimuli presented in the task evoke a response in the LFP in both the OFC and the MFC. This evoked response is composed of a low (<2 Hz) and mid-range frequency band (4-20 Hz). The power in these frequency bands scales with the reward-value of the visual stimuli. These value-induced modulations in LFP are stronger and occur earlier in the OFC than in the MFC. Following bilateral, excitotoxic lesions of the amygdala and, consistent with the single neuron data that were previously reported, the magnitude of the evoked LFP response decreases and the latency of the response is delayed. However, in contrast to the effects of amygdala lesions on single neuron encoding, the number of LFP sites that encode reward-value was significantly reduced in both the OFC and MFC. These analyses reveal that following amygdala lesion there is an uncoupling of LFP and single unit activity related to reward-value coding in MFC. Future analysis will further determine the relationship between single unit activity and fluctuations in LFP power.

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Support: NIDA R00 027718

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Title: Contributions of the orbitofrontal cortex (OFC) to cognitive flexibility and associative learning during an attentional set-shifting task

Authors: *M. D. CASTAGNO, B. J. SLEEZER, B. Y. HAYDEN;
Univ. of Rochester, Rochester, NY
**Abstract:** Flexible decision-making often requires a process of trial and error learning to discover newly relevant rules or stimulus-reward associations. Although numerous studies indicate that different regions of the striatum and frontal cortex contribute to different types of flexible decision-making, few have examined how these regions contribute to different phases of rule identification throughout periods of trial and error learning. Recent work from our lab suggests that the ventral and dorsal striatum differentially contribute to early and late phases of trial and error learning during a primate version of the Wisconsin Card Sorting Task (a classic test of attentional set-shifting). To further examine how the brain implements flexible shifts in attention when trial and error processes are required, we recorded the activity of single neurons in the orbitofrontal cortex (OFC) while two monkeys performed a monkey version of the Wisconsin Card Sorting Task. Our version of the task involved a trial-and-error phase before monkeys could identify the correct rule on each block. Our data indicate that neurons in OFC demonstrate changes in firing rate associated with switching rules (i.e., switch signals). We also find that neurons demonstrate changes in firing rate in response to reward predictive stimuli (i.e. associative learning signals). More specifically, we find that associative learning signals peak early in the block and plateau for the remainder of the block. Taken together with our previous studies in the striatum, these results suggest that the OFC and striatum may work in conjunction to facilitate attentional set-shifting when trial and error learning processes are required. Although these results appear to be inconsistent with studies indicating that damage to or inactivation of the OFC does not disrupt this type of cognitive flexibility, we hypothesize that the OFC may be involved, but not essential for this process. Moreover, we hypothesize that attentional deficits in disorders such as ADHD may arise due to combined dysfunction in the OFC and striatum.

**Disclosures:** M.D. Castagno: None. B.J. Sleezer: None. B.Y. Hayden: None.

**Poster**

**531. Decision Making and Attention: Prefrontal Cortex**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 531.23/Z27

**Topic:** F.02. Animal Cognition and Behavior

**Support:** NIH R01 DA037229

**Title:** Demand for control reduces coding sparseness in dorsal anterior cingulate cortex

**Authors:** *H. AZAB, B. HAYDEN;*
Brain and Cognitive Sci., Univ. of Rochester, Rochester, NY
Abstract: Factors that elicit control, such as conflict and surprise, enhance aggregate neural activity in dorsal anterior cingulate cortex (dACC) but do not consistently drive activity of single neurons. We hypothesized that aggregate measures may reflect broader recruitment of neurons that link contexts to strategies rather than responses of specific neurons that abstractly signal the need for control. In other words, aggregate responses to the need for control may be a consequence, not a direct cause, of control. In a new gambling task, we found that conflicting options and surprising outcomes do not measurably increase neuronal activity, but do increase coding density (meaning, they reduce population sparseness). We propose a simple connectionist model in which all individual dACC neurons link contexts to strategies, and in which practice and familiarity (assumed to be greater to low-conflict and unsurprising outcomes) prunes connections. This pruning leads to more efficient responding but reduced recruitment, and thus lower BOLD signal, for default cognition relative to controlled cognition. These findings endorse the novel hypothesis that dACC regulates control emergently, without the need for specific dedicated control neurons.

Disclosures: H. Azab: None. B. Hayden: None.

Poster

531. Decision Making and Attention: Prefrontal Cortex

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 531.24/Z28

Topic: F.02. Animal Cognition and Behavior

Title: Large-scale sensory integration in the mouse cortex during a tactile detection task

Authors: *P. F. LE MERRE\textsuperscript{1,2}, P.-A. SALIN\textsuperscript{3}, C. C. H. PETERSEN\textsuperscript{1}, S. CROCHET\textsuperscript{1,2}; \textsuperscript{1}Lab. of Sensory Processing, Brain Mind Inst. Fac. of Life Sci., Ecole Polytechnique Federale De Lausanne (EPFL), Lausanne, Switzerland; \textsuperscript{2}WAKING Group, \textsuperscript{3}SLEEP Group, CRNL, Lyon, France

Abstract: Sensory perception leading to goal-directed behavior involves multiple, spatially-distributed and inter-connected cortical areas. It has been hypothesized that sensory information flows from primary sensory areas encoding mainly the properties of the stimulus, to higher-order, more frontal areas encoding the valence of the stimulus. To understand further the integration of sensory signals, we have recorded sensory evoked potentials (SEPs) simultaneously from different cortical areas in mice performing a whisker-based sensory detection task (Sachidhanandam et al., 2013). Mice were chronically implanted with 6 high-impedance electrodes and were either trained to lick a spout immediately after a 1 ms single
whisker deflection to obtain a reward (detection task) or were exposed in the same conditions to
the whisker stimulus that was not associated with the reward (neutral exposition). In trained
mice, we observed SEPs in all recorded areas with latencies increasing from the barrel-field of
the primary somatosensory area (wS1) to the secondary somatosensory area (wS2), the whisker
motor area (wM1), the parietal area (PtA), the dorsal hippocampus (dCA1) and the medial
prefrontal cortex (mPFC). For each area, we investigated whether the sensory evoked responses
correlated with task performance by comparing Hit and Miss trials. We found that the early peak
of the SEP differed little between Hit and Miss trials in most areas except for mPFC and dCA1,
where SEPs in Miss trials were markedly reduced. Interestingly, SEPs recorded in mice after
neutral exposition were also particularly reduced in these areas as compared to trained mice,
suggesting that training induced plastic changes in the mPFC and hippocampus leading to
increased SEPs in response to the conditioning stimulus. Our results support the idea that mPFC
and dCA1 could signal the relevance of a sensory stimulus in the context of a well-defined
behavior, whereas sensory areas would be more constrained by the nature of the stimulus.


Poster

531. Decision Making and Attention: Prefrontal Cortex

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 531.25/Z29

Topic: F.02. Animal Cognition and Behavior

Support: NIDA R00-DA027718

NEI T32-EY007125

Title: Differential contribution of ventral and dorsal striatum to early and late phases of
cognitive set reconfiguration

Authors: *B. J. SLEEZER, B. Y. HAYDEN;
Brain and Cognitive Sci., Univ. of Rochester, Rochester, NY

Abstract: Flexible decision-making, a defining feature of human cognition, is typically thought
of as a canonical prefrontal cortex (PFC) function. However, recent work suggests a possible role
for the striatum as well. We recorded activity of neurons in both the ventral (VS) and dorsal (DS)
striatum while rhesus macaques performed a version of the Wisconsin Card Sorting Test, a
classical test of flexibility. Our version of the task involved a trial-and-error phase before monkeys could identify the correct rule on each block. Our data indicate that neurons in both the VS and DS demonstrated changes in firing rate associated with switching (i.e. switch signals). We found that switch-related activity was strongest in VS early in the trial-and-error period of the block, when the rule was unknown, and strongest in DS later, immediately before the rule was fully established and maintained. We also found that neurons in the VS and DS demonstrated changes in firing rate when monkeys viewed reward predictive stimuli (i.e. associative learning signals). Importantly, we found that associative learning signals arose earlier in the block in the VS and later in the DS, consistent with the appearance of switch signals in each region. These results support a functional hand-off from VS to DS during set reconfiguration and suggest that striatal switch signals may play a role in facilitating the acquisition of stimulus-reward associations during flexible decision-making.

Disclosures: B.J. Sleezer: None. B.Y. Hayden: None.

Poster

531. Decision Making and Attention: Prefrontal Cortex

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 531.26/Z30

Topic: F.02. Animal Cognition and Behavior

Title: Selective inactivation of the nucleus accumbens and the prefrontal cortex during value based decision making in mice

Authors: *H. NAKAYAMA¹, N. HEINTZ¹,²; ¹The Rockefeller Univ., New York, NY; ²Howard Hughes Med. Inst., New York, NY

Abstract: Adaptive decisions require multiple cognitive processes. These cognitive processes include initiating actions at appropriate timing, selecting appropriate actions, and evaluating outcomes to adjust subsequent decision. To understand neural mechanisms underlying these processes, we trained mice to perform a two-option choice task with probabilistic reward in which mice need to learn through trial and error. Mice were required to respond to a nose port in the center to initiate each trial. Subsequently, mice were allowed to make either left or right choice followed by reward delivery at predetermined probability. Each trial was divided into three time windows (trial initiation period, choice period and outcome delivery period), and neuronal activity in the prefrontal cortex (PFC) and the nucleus accumbens was optogenetically inactivated during these time periods. Distinct circuit elements in the PFC and the NAc were inactivated using transgenic mouse lines and AAVs expressing Archaerhodopsin (ArchT). We
found differential behavioral changes depending on the target and the timing of inactivation. During the trial initiation period, inactivation of indirect pathway medium spiny neurons (MSNs) in the NAc increased number of premature response, which was defined as left or right choices without initiating trials with center nose-pokes. A similar change in premature response was also observed after inactivation of specific inputs to the NAc or direct inactivation of the PFC while inactivation of direct pathway MSNs had no effect. During the choice period, inactivation of the PFC affected the current choice. Inactivation of direct pathway MSNs during the outcome delivery period affected the subsequent choice in an outcome dependent manner. Inactivation increased the probability of mice making the same choice in the subsequent trial, and this effect was specifically observed after unrewarded trials. These results suggest that distinct circuit elements in the PFC and the NAc work together for successful task execution and value based decision making.

Disclosures: H. Nakayama: None. N. Heintz: None.

Poster

531. Decision Making and Attention: Prefrontal Cortex

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 531.27/Z31

Topic: F.02. Animal Cognition and Behavior

Support: NSF Graduate Research Fellowship

NIH-NRSA Training Grant 5T32NS041228-13

Title: Ensemble coding of goal-directed actions in the mouse premotor cortex

Authors: *M. J. SINISCALCHI\(^1\), V. PHOUMTHIPPHAVONG\(^2\), M. LOZANO\(^2\), A. C. KWAN\(^2\);
\(^1\)Interdepartmental Neurosci. Program, Yale Univ., New Haven, CT; \(^2\)Dept. of Psychiatry, Yale Univ. Sch. of Med., New Haven, CT

Abstract: The ability to adjust rapidly to different situations is a hallmark of cognitive control. However, the cortical network mechanisms that mediate switching between multiple action selection strategies are unclear. Here, we used head-fixed behavior, two-photon calcium imaging, and pharmacological inactivation to dissect the role of ensemble dynamics in the mouse premotor cortex (M2/Cg1) for strategy switching. We found that multiple behavioral strategies were each associated with a distinct subset of population activity patterns. Interestingly,
subsequent presentations of the same task set returned the network to similar dynamics. These stable yet divergent network states altered choice representation by recruiting different subpopulations of neurons, but did not change the choice preference of individual cells. Additionally, muscimol inactivation of M2/Cg1 facilitated repetitive action selection, while disrupting the transition to a goal-directed behavioral strategy. Our results show that the mouse premotor cortex employs multiple functional configurations to match the behavioral demands of a task requiring cognitive flexibility.

Disclosures: M.J. Siniscalchi: None. V. Phoumthipphavong: None. M. Lozano: None. A.C. Kwan: None.

Poster

531. Decision Making and Attention: Prefrontal Cortex

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 531.28/Z32

Topic: F.02. Animal Cognition and Behavior

Support: NIH grant DA029330

NIH grant MH104460

Title: Neurons in the primate dorsolateral prefrontal cortex encode summed quantities and choices during an arithmetic task

Authors: *B. Massi, H. Sohn, H. Seo, D. Lee;
Neurobio., Yale Univ., New Haven, CT

Abstract: The ability to perform mental arithmetic on quantities provides a fitness advantage to human and non-human animals. For example, an animal may want to estimate the total number of stored food items across multiple caches, or a banker may want to optimize a number of financial investments. Despite the ubiquity of this cognitive process, the neurophysiological basis of mental arithmetic is seldom studied in isolation. To study the neurophysiological underpinnings of mental arithmetic in the dorsolateral prefrontal cortex (DLPFC), a region involved in both numerical cognition and executive control, we developed a novel arithmetic task that requires monkeys to estimate the sum of two quantities. During each trial, the animal fixated a central target while three clusters of dots appeared sequentially around the fixation target. After a short delay, each cluster entered one of the two peripheral target areas, which was followed by another delay before the presentation of the next cluster of dots. The first cluster of
dots (the augend) remained hidden until the end of the trial after it entered the target area. The second group of dots (the addend) appeared centrally and moved to the same target area containing the augend, but remained visible. Finally, the third group of dots (the singleton) appeared and moved to the other target area and also remained visible. After another delay, the animal was required to shift its gaze to one of the targets and received a juice reward with a magnitude proportional to the number of dots at the chosen target. Importantly, the probability that the sum was greater than the singleton was 0.5 for each value of the sum, so the sum of the augend and addend alone could not inform optimal action selection. Overall performance in the arithmetic task was significantly above chance (~83%), even on trials in which non-arithmetic heuristics are ineffective (~76%) and control trials in which numerosity was dissociated from low-level visual cues (~83%).

We found that single neurons in the dorsolateral prefrontal cortex heterogeneously encode task-relevant variables, including the numerosity of the augend and addend, as well as their sum in addition to information about planned actions. These results suggest that the DLPFC contains multiple types of neural signals that are important for arithmetic operations.


Poster
531. Decision Making and Attention: Prefrontal Cortex

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 531.29/Z33

Topic: F.02. Animal Cognition and Behavior

Support: BLBT, MouseNet-30

Title: Strain specific patterns of the mouse brain functional and structural connectivity

Univ. Hosp. Freiburg, Med. Physics, Freiburg, Germany

Abstract: Enormous effort has been focused during the last decade on addressing non-invasively the issue of intrinsic organization of brain functional connectivity (FC). Using resting state fMRI (rsfMRI), the FC architecture of the human brain has been revealed in multiple networks. However, the intrinsic connectional architecture of functional networks (FN) in the mouse brain remains a significantly underexplored research area. The primary goal of our study was to bridge this gap, by systematically and comparatively probing the intrinsic brain FC of two mouse
strains, intensively used in the fundamental and preclinical neuroscience: the C57Bl6/N and the Balbc/J strains. We compared the strain-dependent living mouse brain FC patterns with the structural connectome fingerprints, mapped in-vivo at high resolution diffusion based tractography. We depicted with rsfMRI inter-strain variations in the FC network of the mouse brain. The relevant, influential brain regions were classified for each strain, using the strength and the number of relevant connections. Regions with simultaneous above mean strength and mean number of relevant connections were considered hubs. Some common FC hubs were identified in both strains. This includes, Hippocampus, Cingulate cortex and Retrosplenial (dys)granular cortices as important areas of the limbic system, as well as motor cortex or thalamus which is also known as an important relay for structural connectivity. A first interesting between-strains difference was the inclusion of the somatosensory cortical (SSC) areas as hubs for the C57Bl6/N strain but their exclusion from the group of Balbc/J mice. We further checked if the difference arises from decreased (statistically irrelevant) inter-hemispherical connectivity of the SSC areas or from reduced intra-hemispherical connectivity. Our investigation revealed significantly lower inter-hemispherical correlations between the primary SSC in Balbc/J population when compared with the C57Bl6/N strain. However, a stronger intra-cortical connectivity was assessed in this strain, suggesting a different assembly of the brain networks in the two strains. The modifications into the inter-hemispherical FC were paralleled by the structural networks results in Balbc/J mice, showing important variations in the structural callosal inter-hemispherical pathway. Uncovering the large scale functional and structural connectivity patterns, in a strain specific manner, represents a first step towards a better understanding of modifications in basal, healthy state networks under the impact of various factors, related with genetic, pharmacological or pathological conditions.

Disclosures: L. Harsan: A. Employment/Salary (full or part-time): University of Strasbourg, Faculty of Medicine, ICube, M. Reisert: None. A. Mechling: None. N. Huebner: None. T. Bienert: None. H. Lee: None. J. Hennig: None. D. Elverfeldt: None.

Poster

532. Decision Making: Rodents

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Program#/Poster#: 532.01/Z34

Topic: F.02. Animal Cognition and Behavior

Support: NHMRC Grant #633267

ARC Laureate Fellowship FL0992409
Title: Manipulating projection-specific neuronal populations using a dual viral approach to examine the neural bases of Pavlovian-instrumental transfer

Authors: *B. K. LEUNG*¹, L. S. ZWEIFEL², B. W. BALLEINE³;
¹The Univ. of Sydney, Brain and Mind Res. Inst., Camperdown, Australia; ²The Univ. of Washinton, Seattle, WA

Abstract: Stimuli that predict rewards can have both general invigorating properties and selective response-biasing properties on goal-directed actions. The influence predictive learning has on choice between actions can be demonstrated using the outcome-specific Pavlovian-instrumental transfer (PIT) paradigm; specifically, during the presentation of a conditioned stimulus (CS), rats selectively increase the performance of instrumental actions that predict the same outcome as the CS. We previously demonstrated that disconnection of the medial ventral pallidum (VPm) and the mediodorsal thalamus (MD) removes the response-biasing properties of a stimulus such that rats respond indiscriminately on actions during the presentation of the CS. Here, we employed the use of designer receptors mediated by designer drugs (DREADDs) to target projection-specific neuronal populations during specific PIT. It was revealed that inhibiting VPm axonal terminals in the MD produced a similar deficit to the disconnection such that the rats did not show a bias towards the instrumental action that shared a common outcome with the CS. On the other hand, using DREADDs in combination with the retrograde transducing canine adenovirus-2 virus expressing Cre construct (CAV2), we have demonstrated that activating MD-projecting VPm neurons enhanced the response biasing properties of animals during PIT performance compared to controls, suggesting artificial activation of these neurons facilitated PIT. Finally, we examined regions in the prefrontal cortex (PFC) receiving input from the MD during PIT performance. We employed an inter-hemispheric disconnection approach where two projection-specific neuronal populations were manipulated in opposite hemispheres to evaluate serial connections from the VPm to MD and MD to PFC during PIT performance. Together, these results provide the first evidence of direct, monosynaptic pathways in a serial circuit mediating the response-biasing properties of a CS in specific transfer.

Disclosures: B.K. Leung: None. L.S. Zweifel: None. B.W. Balleine: None.

Poster

532. Decision Making: Rodents

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 532.02/Z35

Topic: F.02. Animal Cognition and Behavior
Support: NIH Grant R01-MH092868
NIH Grant K99-MH104716

Title: Examining the role of the LC in foraging and exploration

Authors: *G. Kane*¹, E. M. Vazey², R. C. Wilson³, A. Shenhab¹, G. Aston-Jones², J. D. Cohen¹;
¹Dept. of Psychology, Princeton Univ., Princeton, NJ; ²Brain Hlth. Inst., Rutgers Univ., Piscataway, NJ; ³Dept. of Psychology, Univ. of Arizona, Tucson, AZ

Abstract: Locus coeruleus-norepinephrine (LC-NE) neurons fire in two modes: phasic, with burst responses to salient stimuli; and tonic, with increased baseline firing and an absence of burst responses. The adaptive gain theory (AGT) proposes that these modes reflect different behavioral strategies - burst responses in the phasic mode orient animals to respond to stimuli that predict rewards, facilitating exploitation of available rewards; but when little reward is available, increased baseline activity in the tonic mode facilitates searching for new sources of rewards (Aston-Jones & Cohen, 2005). However, these predictions have never been directly tested at the neural level. To test these predictions, we used Gq coupled designer receptors exclusively activated by designer drugs (DREADDs; hM3Dq) to stimulate tonic activity in LC neurons in rats performing a patch foraging task and an exploit-explore task. In the foraging task, rats lever press for rewards in a depleting patch, but can choose to incur a time cost to forage to a patch with replenished reward (foraging decision). We found that rats will stay in patches longer when they start with higher rewards, and they will stay in all patches longer when the time to forage to a new patch is increased. AGT predicts that stimulating LC tonic firing will cause rats to leave patches earlier. However, we found that DREADD stimulation of LC-NE neurons increases reaction time to make decisions, increasing the time animals spend in patches. In our exploit-explore task, rats play a series of games in which we examine how they decide between an option with known reward value and one whose value is ambiguous. We found that rats attribute an information bonus, an increase in the perceived value of the ambiguous option for the information gained from selecting that option, which is used to guide future decisions. Additionally, rats exhibit increased decision noise at the beginning of games. Increased decision noise reduces the influence of reward value on decisions, promoting sampling of all options, which also promotes learning the value of the ambiguous option. AGT predicts that stimulating LC tonically should increase decision noise. Tests of this idea are underway. Future studies will more precisely determine how LC influences foraging and exploit-explore decisions. Supported by PHS grants R01-MH092868, K99-MH104716.


Poster
Effects of adolescent cannabinoid exposure on risk based decision-making in rats

Authors: *E. Jacobs-Brichford, L. R. Amodeo, M. S. McMurray, J. D. Roitman;
Univ. of Illinois At Chicago, Chicago, IL

Abstract: Adolescence is characterized by increases in risk-taking across a range of behaviors, including experimentation with alcohol and illicit drugs. Marijuana is the most widely used illicit drug among adolescents, and increased use coincides with a period of marked brain development, particularly in the prefrontal cortex (PFC). PFC is important for responding flexibly and adaptively to variations in environmental contingencies. Perturbations of PFC maturation can lead to impaired decision-making, resulting in increased impulsivity and excessive risk-taking. Of particular interest is medial prefrontal cortex (mPFC), which is continuing to develop throughout adolescence, and is thought to be crucial in shifting behavior as rewards become uncertain or less valuable. Marijuana use during adolescence may impair mPFC function, leading to increased preference for riskier rewards. In this study, we investigated the long-term effects of chronic adolescent cannabinoid exposure on risk based decision-making. 24 male and female Long Evans rats received i.p. injections of WIN 55,212-2, a CB1 agonist, from postnatal day 30-60. Once animals reached adulthood, their risk-preference was measuring using a gambling task. Rats chose between two levers, one of which paid a small, certain reward, and the other paid a larger, probabilistic reward. The probability of receiving the large reward was varied randomly on each session, ranging from 12.5% to 67%. As rats performed this task, we used in vivo electrophysiology to record mPFC activity. Neural responses to cues and outcomes are compared across the range of risk-preferences between WIN-treated animals and controls.

Disclosures: E. Jacobs-Brichford: None. L.R. Amodeo: None. M.S. McMurray: None. J.D. Roitman: None.
Title: Comparison of neural representations between rat anterior insular and orbitofrontal cortex in risky decision making

Authors: *H. ISHII*, Y. KAIZU¹, S. TAKAHASHI¹, S. OHARA¹, P. N. TOBLER², K.-I. TSUTSUI¹, T. IIJIMA¹;
¹Div. of Sys. Neurosci., Tohoku Univ., Miyagi-Ken Sendai-Si, Japan; ²Univ. of Zurich, Zurich, Switzerland

Abstract: We often puzzle over the choice of taking a risk for a higher gain or playing it safe and thereby avoiding a loss. Excessive risk taking can lead to ruin but, we never win races if we always avoid risks. How does the brain balance risk taking and avoiding? The two brain regions, the anterior insular cortex (AIC) and the orbitofrontal cortex (OFC), have been thought to play key roles in this risky decision making (Tobler et al, 2007; Burke et al, 2013). We previously reported that inactivation of the AIC decreased risk preference of the rats in a gambling task whereas inactivation of the OFC increased it (Ishii et al., 2012). These results suggest that the AIC promotes risk taking whereas the OFC suppresses it. They also imply the possibility that the relative strength of the AIC and the OFC activities are crucial for balancing the opposing motives of whether to take a risk or avoid it. To test this hypothesis, we have recorded neural activities of the AIC and the OFC during the performance of the gambling task which we had used in the previous study. The outline of the task is as follows. Water deprived rats were given two choices to get water. One was a sure option which guaranteed 2 drops of water. The other was a risky option which possibly provided 4 drops of water but in 50% (no water in the other 50%). These two options were associated with left and right levers respectively. Interestingly, the rats normally prefer the risky option in this task (Ishii et al., 2012; Ishii et al., 2014), and the rats used in the present study showed consistent preferences. To date, we have recorded single unit activities from 131 neurons in the AIC and 20 neurons in the OFC. Majority of the neurons (over 80%) showed related activities to any event of the task (lever press, outcome announcement, reward delivery, etc.). Among them, here we focus on the neural activities appeared just before the lever press which might relate to the choice of either the risky option or the sure option. We found that 11 AIC neurons showed larger increase in the firing rate for risky choices than for sure choices. These activities were possibly interpreted as risky choice selective, but before that, the following possibility should be excluded. These activities might reflect just subjective preference rather than purely selectivity for risky choice because behaviorally the rats preferred...
the risky option under this choice condition (4/0 drops vs. 2 drops). However, these neurons showed similar selective activities for risky choice under the choice between 4/0 drops vs. 3 drops condition where the rats preferred the sure option. Thus, this type of AIC neuron is thought to be purely “risky choice selective”. A parallel study is going on also on the OFC.


Poster

532. Decision Making: Rodents

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 532.05/Z38

Topic: F.02. Animal Cognition and Behavior

Support: Grant-in-Aid for Scientific Research on Innovative Areas: Prediction and Decision Making (23120007)

Title: Action-dependent state prediction in mouse posterior parietal cortex during an auditory virtual navigation task

Authors: *A. FUNAMIZU, B. KUHN, K. DOYA;
Okinawa Inst. of Sci. and Technol., Okinawa, Japan

Abstract: Model-based decision making requires representation of predicted states that are updated by action-dependent state transition models. To investigate their neural implementation, mice were trained to do an auditory virtual navigation task and neuronal activity was recorded in the posterior parietal cortex (PPC) and its sensory upstream, the secondary visual cortex (V2), with the genetically encoded calcium indicator GCaMP6f after gene transfer by AAV2/1 and 2-photon microscopy. A mouse was head restrained and maneuvered a spherical treadmill. 12 speakers around the treadmill provided an auditory virtual environment. The direction and amplitude of sound pulses emulated the location of the sound source, which was moved according to the mouse’s locomotion on the treadmill. When the mouse reached the sound source and licked a spout, it got a water reward. The task consisted of two conditions: continuous condition in which the guiding sound was presented continuously and intermittent condition in which the sound was presented intermittently. In both conditions, mice increased lickings as they approached the sound source, indicating that mice recognized the sound-source position and predicted a reward. In intermittent condition, the anticipatory licking was increased even when the sound was omitted (Mann-Whitney U-test, p = 4.87E-8), suggesting that mice updated the
predicted sound-source position based on their own actions. We optically recorded calcium transients of up to 500 neurons simultaneously in each of layers 2, 3 and 5 in 8 mice. A subset of neurons increased the activities as mice approached the sound source (i.e., the goal). This increase of activities was observed both with and without sound inputs in all the layers of PPC (two-sided paired t-test, \( p = 0.0049 - 2.42E-11 \)), while the increase was observed only during sound inputs in V2 (\( p = 0.679 - 0.0279 \)). To test how the increase of activities in PPC and V2 contributed to the prediction of goal distance, we conducted a decoding analysis. Bayesian decoder predicted the goal distance from the recorded population activities: the decoder was trained with the data in continuous condition. In layers 3 and 5 of PPC, the predicted distance significantly decreased both with and without sound inputs (two-sided paired t-test, \( p = 2.54E-5 \) and \( 1.91E-5 \)) consistently with the actual distance to the goal. In contrast, V2 required the sounds for updating distance predictions (\( p = 0.0752 \) and \( 0.0412 \)). These results suggest that PPC, but not V2, realizes action-dependent state prediction in the absence of sensory input.

Disclosures: A. Funamizu: None. B. Kuhn: None. K. Doya: None.

Poster

532. Decision Making: Rodents

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Topic: F.02. Animal Cognition and Behavior

Support: Children's Healthcare of Atlanta

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NIH P30NS055077
NIH DA015040

Title: Fasudil, a Rho-kinase inhibitor, transiently remodels prelimbic prefrontal cortical dendritic spines and enhances goal-directed decision-making

Authors: *A. M. SWANSON*\(^1,2\), S. L. GOURLEY\(^1,2\);
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Abstract: An essential component of goal-directed decision-making is the ability to maintain flexible responding based on an expected reward. Doing so requires learning about, and consolidating, information regarding the predictive relationship between actions and their outcomes. Effective consolidation may require plasticity of cortical circuits, including the structural remodeling of dendritic spines. We investigated this hypothesis by inhibiting Rho-kinase, a key regulator of the actin cytoskeleton. First, we show that deep-layer prelimbic prefrontal cortical dendritic spine density predicts decision-making strategies, such that lower densities are associated with engagement in action-outcome response strategies, while higher densities are instead associated with stimulus-response habits, which are insensitive to action-outcome contingencies. Next, we show that pairing the Rho-kinase inhibitor fasudil with an opportunity to update knowledge regarding action-outcome relationships restores goal-directed decision-making in mice that have otherwise developed stimulus-response habits due to extensive response training. This “paired” fasudil delivery results in a transient pruning of prelimbic cortical dendritic spines, while fasudil delivered 4 hours after the event alters neither decision-making strategies nor prelimbic cortical dendritic spine density. These findings suggest that fasudil promotes the consolidation of action-outcome relationships by enhancing the plasticity of dendritic spines in the prelimbic cortex. We next asked whether dendritic spine plasticity is also associated with the consolidation of action-outcome relationships in typical, drug-naive mice. We trained mice to develop either goal-directed or habitual response strategies, and brains were collected either during or after an opportunity to consolidate new information regarding action-outcome contingencies. Dendritic spines in deep-layer prelimbic cortex were enumerated. Together, our findings suggest that action-outcome decision-making is associated with dendritic spine plasticity in deep-layer prelimbic cortex and that enhancing this plasticity, e.g., with fasudil, can augment the consolidation of information regarding the predictive relationship between actions and their outcomes.

Disclosures: A.M. Swanson: None. S.L. Gourley: None.

Poster

532. Decision Making: Rodents

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 532.07/Z40

Topic: F.02. Animal Cognition and Behavior

Support: Princeton University funding to KM

HHMI funding to CB
Abstract: Imagine you are playing chess. As you think about your next move, you consider the outcome each possibility will have on the board, and the likely responses of your opponent. Your knowledge of the board and the rules constitutes an internal model of the chess game. Guiding your behavior on the basis of model-predicted outcomes of your actions is the very definition of cognitive planning. It has been known for many decades that humans and animals can plan (Tolman, 1948), but the neural mechanisms of planning remain largely unknown. Recently, a powerful new tool for the study of planning has become available: the “two-step” task introduced by Daw et al. (2011). This task allows, for the first time, the collection of multiple trials of planned behavior within a single experimental session, opening the door to many new experimental possibilities. We have adapted the two-step task for use with rodents, and developed a semi-automated pipeline to efficiently train large numbers of animals. Here, we show that the rodent two-step task reliably elicits planning behavior in rats, and we characterize the role of the orbitofrontal cortex (OFC) in this planning behavior. We find that inactivations of OFC substantially impair the ability to plan, and that single units in OFC encode planning-related variables, such as the values associated with actions taken at each step in the two-step task. These data demonstrate the OFC is crucial for planning, and begin to shed light on the computational role that it plays in the planning process.

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Poster

532. Decision Making: Rodents

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 532.08/Z41

Topic: F.02. Animal Cognition and Behavior

Support: NIMH Grant 085739

Title: Nucleus accumbens core lesions decrease reward magnitude sensitivity in steady state impulsive choice
Abstract: Impulsive choice behavior involves choosing between a smaller reward that is available after a shorter delay versus a larger reward that is available after a longer delay. Delays or amounts of rewards can be manipulated across conditions to determine general patterns of preference for SS or LL options. A propensity to make impulsive choices has been linked with drug abuse, gambling, and Attention Deficit Hyperactivity Disorder (ADHD). The nucleus accumbens is believed to be critical in determining reward values that guide choice behavior. Research suggests that ADHD may be due to an overly responsive nucleus accumbens core (NAc). Our earlier work with NAc lesions indicated deficits in adjusting to increases in reward magnitude so that when reward magnitude increased, choice behavior did not change significantly. A recent study from our laboratory found that dynamic testing procedures resulted in more random and impulsive behavior. Previous lesion studies employed dynamic procedures which may have shown non-specific deficits in the lesion rats' behavior as a result of dealing with the changing magnitudes and delays. The present study investigated how the NAc influences impulsive choice behavior when using a steady state systematic procedure where opportunities to learn magnitudes and delays is maximized. We hypothesized that rats with NAc lesions should show deficits in adjusting to increases in LL magnitude in comparison to sham control rats if the NAc is critical for reward magnitude valuation. Two groups of rats underwent testing in an impulsive choice task after receiving an NAc neurotoxic lesion or sham surgery. This task exposed rats to a constant short delay (10 s) on the SS lever and a longer (30 s) constant delay on the LL lever. Rats received 1 pellet for choosing the SS in all phases whereas an LL choice resulted in a 1, 2, or 4 pellet reward across the phases. Rats were tested on each magnitude until stable behavior was reached to maximize opportunities for learning of the SS and LL delays and magnitudes. A second test was administered to examine the rats' overall ability to detect differences in reward magnitude using a reward sensitivity task. In this task, both the SS and LL delay utilized a variable interval 30 s schedule (range 1-59 s). An SS choice resulted in a 1 pellet reinforcer and the LL increased across days from 1-4 pellets. Lesion rats showed reduced sensitivity to reward magnitude changes, failing to adapt choice behavior to increases in reward. These results indicate that lesion effects are general across dynamic and steady state tasks, suggesting that NAc lesions result in broad deficits in reward magnitude processing in impulsive choice.

Title: Short- and long-term effects of dietary manipulations on impulsive choice behavior and motivation in rats
Authors: *C. C. HILL, K. KIRKPATRICK; Dept. of Psychological Sci., Kansas State Univ., Manhattan, KS

Abstract: Research in humans suggests that there is a correlation between impulsivity and obesity; specifically, those with higher percent body fat tend to be more impulsive. However, human research is unable to disentangle the cause of greater impulsive choice in people with obesity. For example, obese individuals may make more impulsive decisions as a result of weight gain, impulsive individuals may be at a greater risk for obesity, or some other cause may result in the development of more impulsive behavior. To determine how diet may directly contribute to impulsive choice behavior, the present study manipulated dietary exposure in rats and tested the effects on impulsive choice and incentive motivation. Rats were divided into three groups (high-fat, high-sugar, and control) where diet composition differed, but energy budget remained the same by controlling for the number of calories each group received each day. The high-fat group received chow with a lard supplement, the high-sugar group received chow with a sugar-gelatin supplement, and the control group received all chow. All rats were then tested on a systematic steady state impulsive choice procedure, in which individuals were presented with a choice between a smaller reward available sooner (SS) or a larger reward available later (LL). The LL reward was 2 pellets available after 30 s across all phases. The SS reward was 1 pellet, and it was available after a delay that increased across phases, such that the SS reward was available after 5 s in phase 1, 10 s in phase 2, and 20 s in phase 3. After the impulsive choice task, rats completed a progressive ratio task to evaluate incentive motivation to work for food. Results on the initial impulsive choice task indicated that the high-sugar and high-fat group made significantly more impulsive choices than the control group. After completing the two tasks while on the diet, all rats completed the same tasks again while on an all chow diet. This allowed us to determine if the effects of diet on impulsive choice and motivation were long lasting, or if they reversed after switching to a more balanced diet. The results from this experiment suggest that diet, specifically a diet high in fat or sugar, induces impulsive choice, which may explain the relationship between impulsivity and obesity. These findings present a challenge for promoting healthy food choices, as the food being consumed may make it harder to make the more self-controlled choices that are needed for long-term weight management.

Disclosures: C.C. Hill: None. K. Kirkpatrick: None.
Title: Females in the forefront: the effects of a temporal intervention on impulsive choice in Sprague Dawley rats

Authors: *A. T. MARSHALL, S. L. STUEBING, A. TRIPLETT, K. KIRKPATRICK; Dept. of Psychological Sci., Kansas State Univ., Manhattan, KS

Abstract: Impulsive choice behavior is widely studied in both psychological sciences and neuroscience to better understand the underlying mechanisms of maladaptive behaviors such as gambling, obesity, and addiction. Such experiments often utilize rat models to identify sources of individual differences in impulsive choice. Recent attempts at elucidating the primary mechanisms governing impulsive choice behavior have revealed that individual differences in temporal precision are a significant predictor of individual differences in impulsive choice. Moreover, time-based interventions significantly improve temporal precision and simultaneously reduce impulsive choices. The predominant use of male rats in this research highlights the wide gap in the literature regarding female decision-making behavior, and, as a result, the understanding of mechanisms of impulsive choice in females is limited. The goal of the present experiment was to assess individual differences in impulsive choice in female rats, and determine whether a time-based intervention would reduce impulsive choice, while also improving temporal precision similar to what has been found in male rats. Impulsive choice behavior was assessed through a smaller-sooner (SS) versus larger-later (LL) choice paradigm, in which SS choices resulted in 1 food pellet after 5, 10, and 20 s in successive phases, while LL choices always resulted in 2 food pellets after 30 s. Timing behavior was assessed through peak-procedure trials that were intermixed with free-choice trials. Impulsive choice and timing behavior were comparable to male Sprague Dawley rats from previous studies with these procedures conducted in our laboratory, with both measures affected by changes in SS delay. Half of the rats were then exposed to a temporal intervention which consisted of fixed interval 10- and 30-s schedules of reinforcement, while half of the rats were exposed to a control fixed ratio task. The rats were then re-exposed to the same impulsive choice task as before the intervention. Post-intervention impulsive choice was differentially affected by exposure to the intervention or control task. Overall, this study of female impulsive choice and exposure to
fixed-interval schedules of reinforcement within a temporal intervention aids in understanding the nature of female decision-making processes and suggests some comparable processes to male rats.


Poster

532. Decision Making: Rodents

Location: Hall A

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Program#/Poster#: 532.11/Z44

Topic: F.02. Animal Cognition and Behavior

Support: NIH grant MH-085739

Kansas State Undergraduate Research Award

Title: Social dominance increases risky choice but not impulsive choice

Authors: *J. R. LOTT, C. DAVIS, J. R. PETERSON, K. KIRKPATRICK;
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Abstract: Dominance is a component of social interactions where a dominant individual would have preferred access to an ideal mate, food source, and shelter. Research has shown a dominant/subordinate relationship forms between pair housed rats. Additionally, dominant rats have increased risky decision making, which is the preference for an uncertain larger (UL) reward over a certain smaller (CS) reward in testing. In humans, this translates into a higher incidence of maladaptive behaviors such as drug abuse, gambling, and obesity. However, whether or not impulsive choice is of higher incidence in dominant animals is unclear. Impulsive choice is the preference for a smaller sooner (SS) reward over a larger later (LL) reward. Impulsive choice is also an indicator for similar maladaptive behaviors to risky choice. Another important factor is whether dominance simply enhances the expression of innate behaviors, or if dominance causes those behaviors. The present study therefore sought to assess both risky and impulsive choice as a function of housing and dominance, and examined the effects of current and former housing conditions on choice behavior. The study was conducted in two main parts with different housing conditions. In the first part, half of the rats (n=12) were paired-housed and half (n=12) were singly-housed. All rats then underwent operant response tasks including an impulsive choice task, a risky choice task, and a progressive ratio task (progressively larger
response required for reward). During operant tasks, the rats were measured for dominance via a tube test and pinning behavior in their housing containers. The first part of the study showed no differences between single- and paired-housing conditions in either the impulsive or risky choice tasks. However, dominant rats in the paired housing conditions were significantly more sensitive to probability manipulations in the risky (UL) option and also showed a trend of higher overall risky (UL) choice but no difference in impulsive choice. In part two, the housing conditions were reversed and the same operant tasks and dominance measures were used. Between parts one and two of the study, the behavior of each rat did not change significantly. However, paired rats varied more in their risky and impulsive choice than single housed rats. These results suggest that dominance enhances the expression of innate risky behaviors in paired-housed rats. Variation in behavior caused by paired housing conditions could have potentially important implications for the assessment of individual differences under standard (pair-housed) conditions.

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Poster

532. Decision Making: Rodents

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 532.12/AA1

Topic: F.02. Animal Cognition and Behavior

Title: Flexible use of predictive cues beyond the orbitofrontal cortex: role of the submedius thalamic nucleus

Authors: *M. WOLFF, Dr1,2, F. ALCAZAR2, A. R. MARCHAND2, E. VIDAL2, A. FAUGÈRE2, E. COUTUREAU2; 1CNRS UMR5287, Univ. of Bordeaux, INCIA, Bordeaux Cedex, France; 2CNRS UMR5287, Univ. of Bordeaux, Bordeaux, France

Abstract: The orbitofrontal cortex (OFC) is known to play a crucial role in learning about specific event consequences. However, the contribution of OFC thalamic inputs to these processes is largely unknown. Using a tract-tracing approach, we first demonstrated that the submedius nucleus (Sub) shares extensive reciprocal connections with the OFC. We then compared the effects of excitotoxic lesions of the Sub or the OFC on the ability of rats to use outcome identity to direct responding. We found that neither OFC nor Sub lesions interfered with the basic differential outcomes effect. However, more specific tests revealed that OFC, but not Sub rats were disproportionally relying on the outcome, rather than on the discriminative
stimulus, to guide actions, which is consistent with the view that the OFC integrates information about predictive cues. In a final experiment using a Pavlovian contingency degradation procedure, we found that both OFC and Sub lesions produced a severe deficit in the ability to update Pavlovian associations. Altogether, the submedius therefore appears as a functionally relevant thalamic component in a circuit dedicated to the integration of predictive cues to guide behavior, previously conceived as essentially dependent on orbitofrontal functions.


Poster

532. Decision Making: Rodents

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 532.13/AA2

Topic: F.02. Animal Cognition and Behavior

Title: Thalamocortical control of goal-directed behaviors in rats

Authors: *F. ALCARAZ*, A. R. MARCHAND, E. J. KREMER, E. COUTUREAU, M. WOLFF;

1CNRS UMR5287, Univ. of Bordeaux, Bordeaux Cedex, France; 2CNRS UMR 5535, Univ. of Montpellier, Montpellier, France

Abstract: Survival of living organisms depends on the ability to flexibly select and engage in actions appropriate for their needs or desires. Such adaptive goal-directed behaviors are thought to be supported by distributed circuits encompassing the prefrontal cortex, the striatum, the basolateral amygdala and the limbic thalamus. Recent experimental data consistently indicated that functional interactions between the medial prefrontal cortex (mPFC) and the mediodorsal thalamus (MD) are crucial for these processes. In the present study we detail the anatomical connections between the two regions and provide an explicit functional assessment of this thalamocortical pathway in instrumental tasks. By combining the use of a retrograde viral vector carrying the cre recombinase (CAV-2 Cre) with that of an AAV providing cre-dependent expression of hM4Di (inhibitory DREADD) receptor, we were able to reversibly inhibit the activity of MD neurons selected by their specific cortical projections to the mPFC. While this manipulation has no effect in an instrumental devaluation task, rats were unable to update action-outcome relationships during an instrumental degradation task. Altogether, these results suggest a possible differential involvement of thalamocortical and corticothalamic pathways: the former
appears to be necessary for the flexible use of action-outcome relationships while the later may control outcome value updating.


Poster

532. Decision Making: Rodents

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 532.14/AA3

Topic: F.02. Animal Cognition and Behavior

Support: ANR GOAL

Title: Double dissociation in the role of the basolateral amygdala and the insular cortex in the acquisition and performance of goal-directed actions

Authors: *S. L. PARKES1,2,3, G. FERREIRA1,3, E. COUTUREAU2,3; 1INRA, UMR 1286, Bordeaux, France; 2CNRS, UMR 5287, Bordeaux, France; 3Univ. de Bordeaux, Bordeaux, France

Abstract: Choice between goal-directed actions requires both knowledge of the relationship between actions and their consequences and the ability to compare those consequences on the basis of their current value. Recent evidence indicates that the basolateral amygdala (BLA) and the insular cortex (IC) play a critical role in goal-directed behavior, however the precise role of these regions in the acquisition versus performance of goal-directed actions remains unknown. Here, we used a pharmacogenetic approach, the DREADD (designer receptors exclusively activated by a designer drug), to inhibit neuronal activity in the BLA or the IC selectively during the acquisition or the performance of goal-directed actions. Rats first received stereotaxic injection of an adeno-associated virus expression system carrying the inhibitory hM4Di designer receptor (AAV8-CaMKIIa-hM4Di-mcherry) into either the BLA or the IC. Following recovery, rats were trained to press two levers for two distinct outcomes after which one of the outcomes was devalued by sensory specific satiety immediately prior to a choice extinction test. Rats were injected with either the hM4Di specific agonist clozapine-N-oxide (CNO; 1 mg/kg) or saline either before training or before the choice test. BLA inhibition prior to training, but not prior to test, abolished selective devaluation. In contrast, IC inhibition before test, but not prior to
training, disrupted outcome devaluation. These results suggest a double dissociation in the role of the BLA and the IC in the acquisition and performance of goal-directed actions. Specifically, the BLA, but not the IC, is required for learning specific action-outcome associations whereas the IC, but not the BLA, is necessary for the performance of goal-directed actions based on the current incentive value of their outcomes.

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Poster

532. Decision Making: Rodents

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 532.15/AA4

Topic: F.02. Animal Cognition and Behavior

Support: CIHR MOP-133579

Title: Prefrontal dopamine D1 and D2 receptors regulate dissociable aspects of risk/reward decision-making via distinct ventral striatal and amygdalar circuits

Authors: *N. L. JENNI¹, J. D. LARKIN¹, S. B. FLORESCO²;
¹Psychology, ²Psychology and Brain Res. Ctr., Univ. of British Columbia, Vancouver, BC, Canada

Abstract: We routinely face decisions that require weighing the relative costs and benefits associated with different rewards in order to select better courses of action. Nodes within the mesocorticolimbic dopamine system interact in situations of reward uncertainty to guide risk/reward decisions. Work from our group has shown that D1/D2 receptor modulation within the medial prefrontal cortex (mPFC) increases or reduces preference for large/risky options in opposing ways. Recent work suggests that there are different populations of prefrontal neurons that exclusively express either the D1 or the D2 receptor subtype. Here, we investigated whether these distinct prefrontal D1 or D2 expressing neurons may differentially modulate risk/reward decisions, via distinct subcortical output pathways. Decision-making was assessed using a probabilistic discounting task. Rats were well-trained to choose between levers that delivered small/certain or large/uncertain rewards, the odds of which decreased systematically across 4 blocks of discrete-choice trials (100% - 12.5%). We used asymmetrical unilateral infusions of a D1 or D2 antagonist the mPFC, in combination with a contralateral inactivation of the nucleus
accumbens (NAc) or basolateral amygdala (BLA) to selectively disrupt D1 or D2 modulation of PFC output to these regions. Selectively disrupting D1 modulation of PFC-NAc circuits (but not PFC-BLA projections) reduced preference for the large/risky option, that was driven primarily by a reduction in reward sensitivity, in that rats were less likely to follow receipt of a risky win with another risky choice. In contrast disrupting D2 modulation of PFC projections to the BLA (but not the NAc), increased preference for the large/risky option, driven by a reduction in negative feedback sensitivity, (i.e.; rats were less likely to shift to the small/certain lever following a non-rewarded risky choice). These findings suggest that PFC DA D1 and D2 receptors may modulate dissociable aspects of risk/reward decision-making via actions on separate populations of neurons that have distinct subcortical projection targets. mPFC D1 receptor activity modulates on ensembles projecting to the NAc that promote seeking for larger/uncertain rewards Activation of PFC D2 receptors modulates a separate ensemble projecting to the BLA that are sensitive to non-rewarded actions in order to mitigate biases for the large reward and facilitate flexible patterns of choice. These findings provide novel insight into the dopaminergic microcircuits within the mPFC that facilitate reward seeking and efficient decision making.

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Poster

532. Decision Making: Rodents

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Topic: F.02. Animal Cognition and Behavior

Support: CIHR MOP-133579

Title: Dissociable effects of nucleus accumbens and medial orbitofrontal cortex inactivation and dopaminergic manipulations on risk/reward decision making assessed with a novel “Blackjack” task

Authors: *D. R. MONTES*¹, M. T. L. TSE¹, S. B. FLORESCO²;
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Abstract: Studies with humans and animals have revealed different aspects of risk/reward decision making are mediated by distributed neural circuits that include the pre- and orbitofrontal cortex (OFC), the nucleus accumbens (NAc) and the dopamine system. Previous research from
our group has explored the contribution of these circuits using probabilistic discounting assays. In these tasks, the odds of obtaining a larger reward changes systematically over a session, and rats must keep track of changes in choice/outcome contingencies using internally-generated information to guide efficient decision making. However, real-life choice situations often are associated with external cues that provide some information about the relative likelihood of obtaining reward. For example, an experienced blackjack player knows that the risk of losing a hand is higher when the dealer is showing an “ace” compared to a “6” card. The present study developed a novel operant-based assay we refer to as the “Blackjack” task that models these types of situations. Rats were trained to choose between a small/certain option that always yielded 1 pellet and a large/risky option (4 pellets) delivered with different probabilities. Prior to a choice trial, one of two distinct tones were presented, each of which was associated with either a 12.5% (High Risk) or 50% (Low Risk) chance of obtaining the large/risky reward. An equal number of High and Low Risk trials were randomly interspersed over 40 trials. Under control conditions, well-trained rats selected the risky vs certain option more often (60-70%) on Low Risk trials compared to High Risk trials (30-40%). Reversible inactivation of the NAc shell increased risky choice; an effect that was more prominent on High Risk trials and was driven by a reduction in negative feedback sensitivity (ie reduced lose-shift behavior). In contrast, preliminary data indicate that NAc core inactivation reduced risky choice on Low Risk trials. Inactivation of the medial OFC increased win-stay and lose shift behavior selectively on Low Risk trials, leading to reduced preference for the risky option on these trials. Systemic administration of the dopamine antagonist flupenthixol impaired decision making, increasing/decreasing risky choice on High and Low Risk trials, respectively, whereas amphetamine selectively increased risky choice on High Risk trials, increasing/decreasing win-stay/lose-shift behavior. Collectively, these data provide novel insight into the mechanism that mediate risk/reward judgements guided by external stimuli and suggest that this “Blackjack” task may be useful for investigating the neural bases of normal and abnormal decision making.


Poster
532. Decision Making: Rodents

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 532.17/AA6

Topic: F.02. Animal Cognition and Behavior

Support: JSPS KAKENHI grant number 15K16570
Title: Neural responses observed only in a gambling task in the rat anterior insular cortex

Authors: *Y. KAIZU¹, H. ISHII¹, S. TAKAHASHI¹, S. OHARA¹, P. N. TOBLER², K.-I. TSUTSUI¹, T. IIJIMA¹;
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Abstract: We often puzzle over the choice of taking a risk for a higher gain or playing it safe and thereby avoiding a loss. Excessive risk taking can lead to ruin but we never win races if we always avoid risks. How does the brain regulate risk taking? We are focusing on the anterior insular cortex (AIC) as a key player which influences the risky decision making. We previously investigated the causal role of the AIC in risky decision making by using pharmacological inactivation on the rats (Ishii et al., 2012). In the study, water deprived rats were required to choose either a risky option which possibly provided 4 drops of water but in 50% (no water in the other 50%) or a sure option which guaranteed 2 drops of water. The rats normally prefer the risky option in this task. However, when the AIC was inactivated, the rats decreased their preference for the risky option and came to choose the sure option. From this result, it is concluded that intact AIC promotes risk taking. To reveal the AIC function more clearly we recorded single unit activities from the AIC during the performance of the same gambling task. Here we focus on the neural responses during the outcome delivery. In this task, the rats were noticed whether they got win or lost by presenting different sound before they got reward. We found that a subset of neuron increased the firing rate only when the sound indicating win was presented. Interestingly, these activities disappeared when the risky option was replaced to the sure option. Other subset of neuron responded only to the sound indicating loss. According to the results of the previous studies (Ishii et al., 2012, 2014), the following neuronal activities are expected to be recorded. Some AIC neurons might show the change in the firing rate at the decision period in the risky choice. Some AIC neurons at the waiting period might reflect win expectation/loss anxiety when the risky option was chosen. In the previous studies we found that the current decision is influenced by win/loss-experience in the previous risky choice (outcome history-dependent effect). So, the outcome related activities might influence the choice in the next trial.

Title: Acute and chronic effects of d-amphetamine on decision-making and loss sensitivity

Authors: *S. WONG*¹, C. A. BADENHORST², A. BRIGGS², J. A. SAWADA², A. J. GRUBER²;
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Abstract: Repeated administration of psychostimulant drugs such as amphetamine reduces the ability of animals to learn from reward omission. In this study we examined the effect of both acute and chronic amphetamine administration on rapid choice adaptation following reward omission in rats during a competitive 2-choice task. Loss-driven choice adaptation is demonstrated by a so-called ‘lose-switch’ behaviour, in which animals tend to switch actions on trials following reward omission. We found that acute amphetamine injections immediately prior to the task significantly reduced lose-switch responding in a dose-dependent manner, which is consistent with the prevailing theory that brief pauses in dopaminergic tone signal negative reward prediction errors. In contrast, chronic amphetamine administration (3 weeks, escalating dose) followed by 10-day withdraw caused a significant increase in lose-switch responding, suggesting increased sensitivity to reward omission following repeated drug administration. Previous work in our lab (Skelin, 2014) revealed that the dorsolateral striatum (DLS) is critically involved in innate lose-switch behaviour. Other research (Jedynak, 2007) has demonstrated that repeated methamphetamine administration causes increased spine density in the DLS, and decreased spine density in the dorsomedial striatum (DMS). This may result in the DLS exerting greater control over choice behaviour, thereby increasing the expression of lose-switch behaviour. To further investigate the role of dopamine projections to the DLS in mediating lose-switch behaviour, we optogenetically stimulated neurons in the substantia nigra expressing tyrosine hydroxylase, and assayed the effects on lose-switch behaviour and dendritic morphology in the DLS. Together, these experiments demonstrate opposing effects of acute and chronic administration of amphetamine on loss sensitivity, and suggest a role for the dopaminergic innervations of the dorsolateral striatum in this behaviour.

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Poster

532. Decision Making: Rodents
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Program#/Poster#: 532.19/AA8

Topic: F.02. Animal Cognition and Behavior

Support: NSERC

Title: Neurochemical analysis of biogenic amine neurotransmitters and amino acids associated with countermanding task performance in rats

Authors: *J. BEUK*, G. B. BAKER, R. J. BENINGER, M. PARÉ;

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Abstract: The countermanding task measures inhibitory control by examining the ability to withhold a response to a go stimulus when a stop signal is presented occasionally. Male Wistar rats (N = 12) were trained to respond to a visual stimulus by pressing the lever below an illuminated light for food reward, but to countermand the lever press subsequent to a tone (stop signal) presented in 25% of trials after a variable delay. Modelling of task performance provides an estimate of the length of time needed to countermand responses, the stop signal response time (SSRT). Following countermanding testing, brains were extracted, flash frozen and stored until high performance liquid chromatography (HPLC) with electrochemical detection was conducted to determine levels of the monoamine neurotransmitters dopamine, norepinephrine and serotonin as well as their metabolites in frontal cortex and hippocampus. Levels of bioactive amino acids (alanine, arginine, aspartate, GABA, glutamate, glutamine, glycine, D-serine, L-serine and taurine) were determined using HPLC with fluorescence detection following derivatization. Preliminary observations suggest that some of these neurochemical measures predict inter-individual variability in task performance. In general, the levels of monoamines, as well as GABA, in the hippocampus were positively correlated with SSRT, i.e. the higher their levels, the poorer the inhibitory control. These findings pave the way to identify the contributions of neurotransmitter systems to inhibitory control. Acknowledgements: Supported by NSERC and CIHR.


Poster

532. Decision Making: Rodents
Title: Ventral striatum is necessary for temporal specificity of expectations in dopaminergic reward prediction error signals

Authors: *A. LANGDON*¹, Y. TAKAHASHI², G. SCHOENBAUM², Y. NIV¹;
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Abstract: Firing patterns of midbrain dopaminergic neurons are thought to encode errors in reward prediction and, as such, represent a key component in theories of the neural mechanisms of reward-based learning. Among various structures that project to the midbrain, the ventral striatum is often thought to provide the dopamine system with input that represents current reward predictions, and is sometimes conceptualized as a ‘critic’ that learns expected reward value given the environmental state. A recent study tested the role of the ventral striatum in modulating dopaminergic reward prediction signals by recording activity of putative dopaminergic neurons from the ventral tegmental area while rats with neurotoxic (or sham) lesions of the ipsilateral ventral striatum performed a simple odor-guided choice task [1]. The phasic firing of dopamine neurons in sham-lesioned rats was consistent with signaling a reward prediction error when the timing or size of rewards was altered across blocks of the task. In contrast, dopamine neurons recorded from the lesioned rats did not show the characteristic phasic prediction-error pattern when the timing of reward delivery was changed unexpectedly. Interestingly, the typical prediction-error response to changes in reward size was completely intact, suggesting a functional dissociation between predictions of timing and magnitude of reward. Here we present a model of temporal difference learning that explicitly incorporates temporal expectations in signaling reward predictions, and dissociates these from predictions of magnitude. In this model, temporal relationships between cues and rewards in a given state are learned concurrently with the expected value of that state. Importantly, temporal expectations regarding state duration are used to gate value-based prediction error signals, modulating learning according to the likely time of reward delivery. We model lesions of the ventral striatum as an inability to learn precise temporal expectations regarding reward delivery, and show how this critically changes state value estimation and thus reward prediction-error signals, mimicking the experimental results above. This model makes precise the role of temporal expectations in shaping reward prediction errors and suggests the ventral striatum is critical for forming and exploiting these temporal expectations during reward-based learning. [1]. Takahashi, Y. and Schoenbaum, G. Ipsilateral ventral striatal lesions disrupt reward prediction error signals in rat
The role of Akt1 in the regulation of behavioral and electrophysiological responses in reward-based decision making in mice

**Authors:** *C. CHEN*1,2, Y.-W. LIU2, W.-S. LAI2,3,4; 1Psychology, Lab. of Integrated Neurosci. and Ethology, Taipei, Taiwan; 2Psychology, 3Grad. Inst. of Brain and Mind Sci., 4Neurobio. and Cognitive Sci. Ctr., Natl. Taiwan Univ., Taipei, Taiwan

**Abstract:** Accumulating evidence from human genetic studies and animal studies suggests the involvement of AKT1 in the pathogenesis of schizophrenia and reward prediction error. Recent findings from our lab and others also imply the importance of AKT1 in the regulation of dopamine-dependent behaviors, methamphetamine-induced behavioral sensitization, goal-directed behavior, decision making, and striatal neuronal activity, especially in Akt1-deficient mice. To further investigate the role of Akt1 in the regulation of probabilistic reward-based decision making, male Akt1 heterozygous (Het) mutant mice and their wild-type (WT) littermate controls were used in this study. Both Het and WT mice were trained to learn Pavlovian-conditioned pairing and a 2-choice dynamic foraging task with different reward probability. During the Pavlovian-conditioned pairing, our behavioral data revealed that there is no genotypic difference in their sign-tracking or goal-tracking behavior. However, in the 2-choice dynamic foraging task, Het mice took fewer trials to steadily choose high reward choice compared with WT controls. Their trial-by-trial choice behaviors were further analyzed to elaborate parameters for...
reinforcement learning. Compared with WT controls, our model-fitting data revealed that Het mice have a higher learning rate but a lower choice perseveration. Based upon these findings, in vivo local field potential recording from the dorsomedial striatum was conducted in these mice during different stages of decision making process in the dynamic foraging task. Our preliminary data revealed a genotypic difference in the power spectrum density (dB) of local field potential during baseline. Data collection and analyses are still in progress. Findings from this study can enhance our understanding of the role of Akt1 in reward-based decision making and its involvement in the pathogenesis of schizophrenia.


Poster

533. Executive Function: Neurophysiology

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 533.01/AA11

Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant EY012135

Title: Activity encoding spatial working memory in macaque frontal cortex is highly structured, yet incompatible with current attractor network models

Authors: *C. D. HOLMES, C. PAPADIMITRIOU, L. H. SNYDER;
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Abstract: Working memory, the ability to maintain and transform information, is critical for cognition. Neuronal activity in dorsolateral prefrontal cortex (dPFC) and frontal eye fields (FEF) is elevated while monkeys hold a spatial location in memory for 1-3 s (Bruce and Goldberg, 1985; Funahashi et al. 1989). The premier model for spatial memory is the continuous attractor network (Wang, 2009). Memoranda are represented as localized "bumps" of activity in a topographic map of nodes. Due to a balance between local excitatory and global inhibitory recurrent feedback, the bumps are maintained indefinitely, even after the original stimulus is removed. The amplitude and profile of the bump does not change over time, although random drift leads to inaccuracy that tends to increase with time. Other studies have argued for more complex dynamics in memory activity. For example, single cell responses may ramp up or down over the memory period, or turn on for only a limited time within the memory period. These results have inspired alternatives to the attractor model. We recorded 161 neurons in dPFC and FEF as monkeys held spatial memories for up to 15 s. Single cells were carefully isolated and...
optimally driven, using a continuous distribution of targets. Cells with significant memory activity became tuned early (within 1 or 2 s) and most (80%) lost their tuning (turned off) before the end of the 15 s memory period. Across trials and cells, the time at which cells lost their tuning was exponentially distributed. Surprisingly, however, each cell had a relatively fixed turn-off time. Once off, cells did not turn back on. These results are not compatible with most previous models of memory. While our results are quite novel, they nonetheless replicate the main findings of earlier work that tested memory for only a few seconds. Like the earlier studies, we find that many cells are continuously active during the first two seconds of memory, with activity in some cells ramping up and others ramping down. Transient activations, multiple on/off cycles, and other complex dynamics similar to those reported previously also appear in our data, but only when non-optimal stimuli are used. These complex dynamics do not reflect the operation of memory circuits. Instead, they reflect noisy responses from the flanks of the tuning curves. In summary, most memory cells turn on early in the memory period, stay active for a variable but cell-specific amount of time, then shut off and stay off for the remainder of the memory period. These findings challenge the attractor network model of working memory, but also show that memory responses are much more structured than suggested by competing heterogeneous models.


Poster

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Topic: F.02. Animal Cognition and Behavior

Support: NIH DC04845

NIH EY007125

Title: Involvement of medial prefrontal neurons during the decision process in an audiovisual working memory task

Authors: *B. PLAKKE, L. M. ROMANSKI;
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Abstract: The medial prefrontal cortex (mPFC) is involved in detecting conflict, decision-making, audio-vocal control and motor processing. It also receives input from temporal lobe
auditory and multisensory regions, however few studies have examined its response to naturalistic auditory or audiovisual stimuli. Our previous work has shown that ventrolateral prefrontal cortex (VLPFC) integrates face and vocal information and is necessary for audiovisual working memory. Here, we asked whether mPFC might also play a role in audiovisual processing. We recorded from mPFC in macaques while they performed an audiovisual nonmatch-to-sample task. Subjects attended an audiovisual movie clip of a face-vocalization as the Sample and detected the occurrence of a Nonmatch (when the face or vocalization differed from the Sample movie). Preliminary data showed mPFC cells are active during several task epochs including the sample (55%), the delay (65%), the match (41%) and the nonmatch (32%) period. In contrast to previous findings from VLPFC, a larger percentage of units were responsive during the Match, when the animal correctly withheld a response. Additionally, there was a subset of neurons active during the correct detection of both Nonmatch and Match stimuli, indicative of decision related activity. These decision related units were more commonly found in mPFC compared to previous results from VLPFC. This data suggests that both VLPFC and mPFC are part of a neuronal circuit underlying audiovisual working memory in the primate brain.

Disclosures: B. Plakke: None. L.M. Romanski: None.

Poster

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Prop. 63, the Mental Health Services Act

Behavioral Health Center of Excellence at UC Davis

Title: Transient synchronizations and cross-frequency interactions between prefrontal cortex and striatum underlie category learning

Authors: *R. LOONIS*1,2, E. G. ANTZOUNATOS1, S. L. BRINCAT1, E. K. MILLER1;
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Abstract: There is emerging evidence that oscillatory synchrony plays a role in learning. Thus, we examined oscillations in two areas known to be important for category learning, the prefrontal cortex (PFC) and striatum (STR; Antzoulatos and Miller, 2011 and 2014). Two monkeys were trained, on each day, to classify dot patterns into two categories. Brincat and Miller (2015) reported frequency-specific interactions between the PFC and hippocampus (HIPP) during object associative learning. They found an increase in post-decision beta synchrony (12-30 Hz) if the monkey’s choice was correct and an increase in theta (3-7 Hz) if the choice was incorrect. Similarly, we found increased synchrony between the PFC and STR during category learning but the opposite pattern from that seen between the PFC and HIPP. Beta band synchrony increased after an incorrect choice and theta band synchrony increased after a correct choice. This could reflect a difference between areas or between types of learning (associative vs category). Furthermore, post-decision PFC-STR synchrony in the beta band waxed and waned periodically and was correlated with theta-band evoked potentials in the PFC and STR. This correlation between the PFC-STR synchrony and theta band evoked potentials dissipated over learning. Moreover, during learning, the phase of PFC theta signals modulated the amplitude of gamma (30-50 Hz) activity in the STR. These data suggest that rhythmic interactions between the PFC and STR play a role in category learning.


Poster
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Topic: F.02. Animal Cognition and Behavior

Support: CONICYT, FONDECYT/Postdoctorado 3140306 to CD

Title: Oscillatory synchrony and working memory updating in the monkey cortex

Authors: *C. Devia1,2, J. Rose1,2, E. K. Miller1,2;
1Picower Inst. for Learning and Memory, 2Dept. of Brain and Cognitive Sci., MIT, Cambridge, MA
Abstract: Volitional control of what is maintained is a defining characteristic of working memory (WM), but little is known of its neural basis. To study this, we recorded local field potentials (LFPs) from parietal (LIP) and prefrontal cortex (LPFC and OFC) while subjects performed a value-based working memory task. The monkeys were motivated to retain high-value targets at the expense of low-value targets. On each trial, two targets were presented in sequence at two of four possible locations. Each target was followed by a reward cue that signified the value of that target (juice amount). On some (RETAIN) trials a high target value was followed by a low value target, thus motivating monkeys to retain the first target. On other (UPDATE) trials, the low value target appeared before a high value target, thus motivating monkeys to update the working memory with the second, higher-value target. At the trial’s end, monkey made a saccade to either target location, for which they received that amount of juice.

We compared LFPs power and phase synchrony on RETAIN vs UPDATE trials during the presentation of the second cue. The LIP showed broadband activity, with significantly more power only for UPDATE trials. These episodes of high power spanned from alpha to low gamma band (8-51 Hz). In frontal areas, only low frequencies had significant modulation (2-33 Hz). The LPFC had more power in beta band for UPDATE trials and in delta/theta for RETAIN. We observed the opposite relation for OFC, with significant augmented power in theta range for UPDATE trials and more beta activity for RETAIN. Synchrony between parietal and frontal areas increased, but only for UPDATE trials. The three regions showed increased between-area synchrony in beta and gamma and LIP and frontal areas showed increased lower frequency (2-30 Hz) synchrony for UPDATES. This suggests that oscillatory activity is involved in both updating and protecting (retaining) WM. The LIP is more involved in updating. The prefrontal cortex is engaged for both updating and retaining WM contents.

Disclosures: C. Devia: None. J. Rose: None. E.K. Miller: None.

Poster

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Topic: F.02. Animal Cognition and Behavior

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JFDP Post-Doctoral Fellowship

Title: Temporal fine-structure of activity in PFC of macaque during in multi-item working memory
Abstract: Prefrontal cortex (PFC) neural activity is thought to underlie the maintenance of information in working memory (WM). This activity is often assumed to be more or less continuous, but a sub-class of WM models suggests that the activity occur in discrete bursts. Here, we analyze data from PFC of macaque monkeys performing a multi-item WM task in which items are presented serially to test these assumptions. Neuron spiking as well as LFPs were analyzed. We found stimulus-load related changes in beta (20-37 Hz) and gamma (55-90 Hz) band power in the trial-averaged spectral content. A detailed analysis of the time-frequency spectral content of individual trials suggested a complex dynamic with discrete “bubbles” of elevated gamma or beta power, well defined in frequency and time. These gamma and beta bubbles lasted on the order of 50-300 ms and occurred seemingly at random points during individual trials, but gamma bubbles were predominant during stimulus encoding. By contrast, beta bubbles were more likely during the memory delays. Also, gamma bubbles increased around the time monkeys anticipated having to use the contents of WM and thus may also be important for decoding information from WM. Neuron spiking tended to occur within the gamma bubbles, but the gamma bubbles were not explained by spiking per se because individual bubbles were narrow in frequency range. Thus, spikes seemed to ride on top of gamma bubbles consistent with the view that they reflect brief moments of elevated rhythmic firing in local cell assemblies. These results differ from a standard view of WM in which neurons simply sustain their activity. They support models in which information is stored in WM in discrete bursts of rhythmic firing allowing maintenance of multiple items without interference between them or from sensory processing (Lundqvist et al., 2011). Lundqvist M, Herman P, Lansner A (2011) Theta and gamma power increases and alpha/beta power decreases with memory load in an attractor network model. Journal of Cognitive Neuroscience 23: 3008-3020.

Title: Prefrontal coding shifts from perception to memory recall during learning

Authors: *S. L. BRINCAT, E. K. MILLER;
The Picower Inst. for Learning and Memory and Dept. of Brain and Cognitive Sci., MIT, Cambridge, MA

Abstract: How do neural networks support both bottom-up processing of perceptual inputs and top-down recall of perceptual memories? We simultaneously sampled spiking and local field potential (LFP) activity from the lateral prefrontal cortex (PFC) and hippocampus of monkeys performing an object paired-associate learning task. On each trial, a sequence of two unrelated objects was shown, separated by a brief delay. The monkeys' task was to learn through trial-and-error which objects were arbitrarily designated as associative pairs. Each day, they learned four novel associations within a few hundred trials. PFC spiking activity conveyed both perceptual information about the objects, as well as mnemonic information reflecting anticipatory recall of the expected paired-associate. This mnemonic information increased in parallel with learning, along with a concomitant decrease in perceptual information. Thus, the PFC shifted from emphasizing bottom-up perception to top-down recall. Task performance was also accompanied by prominent beta-band (~12-25 Hz) LFP oscillations, with distinct components tightly locked to object onsets (evoked potentials) and not phase-locked to trial events. Beta-band evoked potentials—likely reflecting bottom-up input—decreased with learning. In contrast, non-phase-locked beta oscillations—likely reflecting local or top-down processes—increased both in their power and in their strength of synchronization with the hippocampus. These results suggest that prefrontal cortex plays a role in both bottom-up perceptual and top-down mnemonic processes. As associative memories are successfully formed, PFC and associated networks appear to switch from a perception-dominated mode to a memory-dominated one.

Disclosures: S.L. Brincat: None. E.K. Miller: None.

Poster

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Location: Hall A

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Abstract: Impulsivity, the tendency to make decisions and/or take action rapidly without long-term foresight, is typically associated with attention-deficit hyperactivity disorder and various forms of drug addiction. Top-down cognitive control mediated by prefrontal cortical interactions with limbic structures, including the hippocampus, is hypothesized to regulate impulsivity. Hippocampal neurons fire during the delay in delayed-nonmatch-to-sample tests or other working memory tasks in monkeys and rodents. This neuronal activity is interpreted to be a mechanism for working memory to hold information for a certain amount of time. It is not clear, however, if the hippocampal neurons encode information for delay discounting of reward values, involved in impulsivity. Using silicon probes with multichannel electrodes, we recorded neural activity in the CA1 region of mice performing a delay-based decision-making task in a T-maze. Large and small rewards were allocated to each side arm with or without a delay, respectively. The delay duration in the large-reward arm was varied (0 - 40 s) in each session. The choice ratio to the large reward-delayed arm was negatively correlated with the delay duration. Local field potentials during the delay were characterized by strong theta oscillations and a lack of sharp-wave/ripples, unlike local field potentials observed during delays in working memory tasks. Sizable numbers of CA1 neurons exhibited significant increases (30% of the population) and decreases (50%) in their firing rates during long (>20 s) delay periods. Importantly, 40% of the delay-activated neurons exhibited significantly increased firing rates as a function of delay length, indicating that these neurons encode information of the duration the mice must wait during the trial. To investigate the location-selectivity of these delay-activated neurons, we switched the large reward-delayed arm with the small reward-non-delayed arm, and found that 80% of the delay-activated neurons had location selectivity and 20% had no location selectivity, indicating that some population of hippocampal neurons encodes waiting times independent of spatial information. These findings suggest that the hippocampus senses a delay and encodes the information required for delay discounting of reward values.

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Poster

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Topic: F.02. Animal Cognition and Behavior

Support: JSPS KAKENHI, Scientific Research (B) #25291071

JSPS KAKENHI, Challenging Exploratory Research #26650114

Title: Computation of reward prediction error by projections from medial striatum to midbrain dopaminergic neurons in domestic chicks

Authors: *C. WEN*¹, T. MATSUSHIMA²;
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Abstract: Midbrain dopaminergic (DA) neurons have been supposed to represent the reward prediction error (RPE) signal, a critical variable in the paradigm of reward-dependent reinforcement learning (Schultz et al. 1991; Roesch et al. 2007; Cohen et al. 2012), but the neuronal mechanisms underlying its computation are not fully understood. Medial striatum (MSt, homologous to the ventral striatum in mammals) may be critically involved, as (1) it has reciprocal connections with DA neurons (Bálint et al. 2004, 2011), and (2) its lesion impairs both acquisition and extinction in food-reinforced associative learning (Izawa et al. 2001, Ichikawa et al. 2004). The following lines of evidence further support the assumed involvements. First, some MSt neurons code the actual reward, whereas other MSt neurons code the reward prediction even in the reward period. Week-old chicks were trained in color discrimination task reinforced by food reward, in which LED colors were associated with foods of different amount and delay. After chicks had been successfully trained, we compared the single unit activities between two conditions. In control condition, chicks received food as trained (therefore, as predicted). In the following omission block, the food was omitted for one of the reinforced LED colors leading to a negative prediction error. MSt neurons were categorized to 2 groups, one with excitation and the other with inhibition during the reward period. Majority of the former (excitation-type neurons, 9/11) stopped firing immediately after the omission. On the other hand, more than half of the latter (inhibition-type neurons, 3/4) maintained inhibition after the omission. In contrast, as the second line of the evidence, considerable proportion of neurons in the midbrain tegmentum coded the negative RPE in reward extinction. The reward-period activity was lower in the omission block, if compared with the activities in the no-reward-predicting trials. These neurons were located in/near the tegmental regions rich in DA neurons. As the third line, MSt neurons proved to have direct synaptic contacts with DA neurons. Anterograde tracing using biotinylated dextran amine revealed terminal boutons juxtaposed on soma and proximal dendrites of the tyrosine hydroxylase immunopositive neurons in the tegmentum. We can reasonably assume a scenario that (1) parallel descending projections from MSt convey both of the reward prediction and the actual reward signal, and (2) summation of these signals yields RPE in DA neurons through direct synaptic convergence. Further in vitro slice studies are needed to characterize how synaptic actions are integrated in DA neurons.
Title: Monkeys (Macaca mulatta) represent magnitude with flexible spatial mappings

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\(^3\)Bucknell Univ., Lewisberg, PA

Abstract: Humans often activate a mental representation of a number line when performing quantitative tasks. This is illustrated in the spatial numerical association of response codes (SNARC) paradigm in which people report whether a sample number is odd or even (Dehaene, Bossini, & Giraux, 1993). Western humans respond more quickly on the left when the number is small and on the right when the number is large. This indicates that responding is facilitated when the response is congruent with the mental number line even though no explicit magnitude processing is required. The polarity of the mental number line is determined by experience reading and counting. This is indicated by the fact that Palestinians, who read and count from right to left, show a pattern opposite that seen in most Westerners, responding faster on the right for small quantities and left for large quantities (Shaki, Fischer, & Petrusic, 2009). The capacity to represent both space and quantity is available to many species, so we might expect similar space-magnitude interactions in nonhuman primates (Adachi, 2015; Rugani, 2015). Monkeys and humans share homologous brain regions implicated in both the processing of space and magnitude. Neurons in the nonhuman primate ventral intraparietal area (VIP) of the intraparietal sulcus, respond selectively to presentations of numerosities (Nieder & Miller, 2004). Single cell recordings from the VIP in macaques have also shown that neurons in this area respond to spatial cognition (for a review see Grefkes & Fink, 2005). The intraparietal sulcus has also been implicated in both spatial and numerical cognition in humans (Hubbard et al., 2005). To determine whether spatial representations are involved in quantity processing across species, we tested for SNARC phenomena in monkeys. Monkeys matched the color of sample arrays of 1 to
10 dots to color comparison stimuli on the bottom left and right sides of a computer-controlled touchscreen. After being trained to associate small magnitude stimuli with left side responses and large magnitude stimuli with right side responses, monkeys were more accurate in color matching when responding on the left after seeing a small number of colored dots and when responding on the right after seeing a large number of colored dots. This occurred even though the number of dots was irrelevant to the task. The effect reversed twice after monkeys were trained in the opposite spatial association. These results are consistent with a flexible spatial representation of magnitude in monkeys.


Poster

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Topic: F.02. Animal Cognition and Behavior

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Title: Two types of representations for numerosity ‘zero’ in the Parietal Cortex of the Monkey

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Abstract: Zero is a fundamental concept in mathematics and modern science. Mathematical history suggests that before the development of the notation system of zero, humans seemed to recognize the concept of zero without a symbol. Recent reports showed that preschool children and non-human primates could distinguish empty objects from existing objects. Therefore, the concept of zero seems to be non-verbal as well as non-zero numerosities. How is numerosity zero (the absence of visual items) represented in the primate cortex? To address this question, we trained two monkeys to perform a numerical operation task including numerosity zero. The monkeys were required to convert a preoperational number of visual objects into a target number of visual objects by numerical operations (addition or subtraction). Behavioral results showed that the monkeys’ success rates linearly declined (linear regression, r² = 0.97, P < 0.01) as a function of the target numerosity including numerosity zero. We recorded 614 cells in the VIP of a monkey performing the task. In the VIP, cellular activity selective to the target numerosity 0-4 was found in 185 neurons (30%) during the presentation of the target numerosity. Among these,
137 neurons showed differential activities to numerosity zero ('zero' neuron) (target period, 99/614, 16%). We found two types of 'zero' neurons in the VIP exhibit changes in activity during the presentation of the target numerosity of zero. The first type of 'zero' neurons (66/614, 11%) exclusively represented numerosity zero in absence or presence manner. The second type of 'zero' neurons (31/614, 5%) encodes numerosity zero and non-zero numerosity continuously (decreased activities as a function of target numerosities). Our findings suggest that numerosity zero could be coded parametrically as an extension of non-zero numerosities on the same numerical continuum (i.e., 0, 1, 2, 3, ...) and exclusively as nothingness of counting objects (i.e., absence or presence). Representations of numerosity zero in the parietal cortex may be a precursor of non-verbal concept of zero in primates.

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Poster

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Topic: F.02. Animal Cognition and Behavior

Support: NSF GRFP

NDSEG

NIMH XXYYZZ

Title: Medial prefrontal cortex supports reversal learning by facilitating shifting amongst competing attractor states in the hippocampus

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¹Neurosci., Mount Sinai Sch. of Med., New York, NY; ²Neurosci., Ichan Sch. of Med. at Mount Sinai, New York, NY

Abstract: Episodic memories reflect the past and inform future behavior. Although these representations can be activated by environmental cues, sensory information alone is often not enough to unambiguously retrieve specific memories. We examined how interactions between the medial prefrontal cortex (mPFC) and the hippocampus contribute to selective memory retrieval as rats learned rapid serial spatial reversals on a plus maze. We found that the mPFC is crucial for disambiguating recent memories, but with an unexpected time course: mPFC activity was necessary during current learning to reduce interference with future learning, but not during
current learning to reduce interference from recent memories. Simultaneous recordings from the mPFC and hippocampus during reversal learning revealed that while population activity of both mPFC and CA1 discriminate goal states, the trajectory of the mPFC representation during reversal learning tends to lead and predict the trajectory of the CA1 representation. CA1 unit recordings in ipsilateral mPFC-inactivated animals showed that the goal representation in CA1 was less distinct across initial learning and reversals and that transitions between states were slower. Taken together, the data suggest that rule representations from the mPFC may be convolved with hippocampal representations in order to help discriminate amongst multiple, recently activated memories; when these discriminators are missing across multiple learning epochs, proactive interference builds up and deficits in reversal learning begin to emerge.


Poster

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JSPS Postdoctoral Fellowships for Research Abroad (KW)

Title: Neural activity of monkey prefrontal and posterior parietal cortex during foraging for multiple targets

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Abstract: The prefrontal cortex (PFC) and posterior parietal cortex (PPC) which are reciprocally connected have been reported to show similar neuronal activity patterns while monkeys perform a memory-guided task. To address roles of the PFC and PPC in working memory for multiple concurrent targets, monkeys were trained with spatial and object foraging tasks in which animals explored a choice array searching for targets and remembered them once found. The target was defined by the location within a 5-choice array in the spatial foraging task, and by the object identity within a 4-choice array in the object foraging task. A choice array was presented on a
touch panel with a central fixation point (FP). Each trial started when the animal held a home key and fixated the FP for 700-1500ms (pre-choice period), then the animal released the home key and touched one of the choice locations /objects when FP changed in color (response period). After 350-450ms of touch-hold period, the color of the selected choice location/object changed to green or red, revealing that location/object was a target or non-target, respectively (feedback period). When a target was selected, a drop of soft food was given as a reward (reward period). The number of targets was one or two and trials were repeated until the animal had touched all the target locations/objects, then a cycle of foraging trials ended. Cycles with the same targets were repeated four times to form a set of cycles, so that targets searched for targets by trial and error in the first cycle (acquisition phase), then exploited this knowledge in subsequent cycles (retention phase). In the acquisition phase, the animals searched targets efficiently with few repeated touches on the same locations/objects. In the retention phase, the animals’ performance improved quickly with almost no error touches in the later cycles. While animals performed the foraging task, we recorded single unit activity in the PFC and PPC. We found many neurons in both PFC and PPC showing selectivity for the chosen location/object in the response period. A smaller number of neurons in both areas showed selectivity in the pre-choice period. In the feedback period, both PPC and PFC also strongly encoded the chosen location/object identity. In addition, the neuronal activity in this period was modulated by the phase, with some neurons more activated in the acquisition phase and others in the retention phase. The results suggest collaboration of PFC and PPC in successive stages of complex, multi-step behavior, including learning from feedback during initial exploration, and use of this knowledge in later exploitation.


Poster
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KAKENHI 26112009
Title: Cytoarchitectonic information of rat medial prefrontal “delay” neurons revealed by single-neuron electroporation

Authors: *K. OYAMA*¹, Y. TATEYAMA¹, C. LO¹, S. OHARA¹, F. KARUBE², F. FUJIYAMA², T. IIJIMA¹, K.-I. TSUTSUI¹; ¹Grad. Schol. of Life Sci., Tohoku Univ., Sendai, Japan; ²Lab. of Neural Circuitry, Doshisha Univ. Grad. Schol. of Brain Sci., Kizugawa, Japan

Abstract: We have recently established a novel technique for labeling single neurons persistently without microscopic guidance by injecting a plasmid encoding a fluorescent protein electroporatively after recording their activity extracellularly with a glass-pipette electrode. This technique enables us to clarify the cytoarchitectonic information of electrophysiologically recorded neurons, such as cell type, laminar distribution, and projection sites. In this study, we investigated the cytoarchitectonic information of rat medial prefrontal cortex (mPFC) neurons which showed the sustained activity during a delay period in a delayed response task, which is thought to be the neural correlate of the working memory, by using this technique. We trained head-fixed rats to perform a visuo-spatial delayed response task. In this task, a visual stimulus (illumination of a LED) was presented at either left or right side of the head of the rat, and the rat was required to lick one of the two spouts at the visually instructed direction after 2 s of a delay period. After they learned the task, we recorded single-unit activity in the rat mPFC during the performance of this task. We found that some mPFC neurons showed sustained activity during the delay period. Immediately after the recording, we switched the connection to the electrode from an amplifier to a stimulation isolator, and injected the plasmid encoding fluorescent protein included within the glass-pipette electrode into the recorded cell by applying a negative voltage pulse train (0.5-ms-long square pulses with an amplitude of −10 V, 50 delivered at 50 Hz). By histological inspection following the recording experiments, we could observe that some neurons were clearly stained with the fluorescent protein, and identify the cytoarchitectonic information of those neurons. We found that stained neurons were mainly pyramidal neurons and that they were distributed both in the superficial and deep layers of the mPFC. We could also trace the axonal projections of some neurons. We found that some neurons in the layer II/III projected to adjacent brain regions (M2 cortex and prelimbic cortex), and some neurons in the layer V projected to the dorsomedial part of the striatum. These results suggest that pyramidal neurons located in various layers in the rat mPFC are involved in the working memory, and support the idea that the delay activity is maintained through the cortico-cortical network and cortico-basal ganglia loop.


Poster

533. Executive Function: Neurophysiology
Title: New insight on neural substrate at single unit level of behavioral task learning from emerging neural activity patterns

Authors: *J. SI, W. MA; Arizona State Univ., Tempe, AZ

Abstract: The goal of this study is to discover the evolution of dynamic single unit based neural activity patterns as task learning takes place. Toward this goal, 1) we collected frontal cortical single unit firing activity from rat medial agranular (AGm) and lateral agranular (AGl) areas while rats performed a direction-cued lever press learning task; 2) we developed a new, automatic, expectation-maximization (EM)-based analysis tool to let neural activity data self-organize and form automatic clusters according to similarities among neural activity patterns. The algorithm, referred to as the automatic and robust EM or AREM, allowed us to capture latent features within the neural activity data automatically and according to objective measures as task learning proceeded. The learning task required rats to associate a left/right side light cue with a respective side lever press. The rats had 2 seconds to choose between a left or a right side lever after the directional cue presentation. It usually took rats 20-30 sessions (days) to learn the task from chance level accuracy to about or above 85% accuracy. We used 16-channel single unit extracellular recording systems to acquire rat neural activity. For all 4 rats analyzed in this study, 4-5 channels from each rat (totaling 19 channels for 4 rats) consistently carried single unit neural action potentials throughout the learning process and therefore were used in this analysis. A single neuron was first sorted and labeled. Its activity during an initial sub-interval (0.8 s) of the cue-on period (2 s) was binned (40 ms bin) to form a vector for each trial. Single session based trials were then pooled together to form the “session dataset”, which were automatically placed into 2-3 clusters. We analyzed the compositions of each cluster through learning. We discovered the following. 1) During initial task learning, all 4 rats encoded previous trial outcome (success or failure). 2) As learning progressed, a dominant cluster emerged, which encoded more strongly the error/success factor than the direction cue/press factor (p<0.05). The other cluster(s) shrank to reach a steady state not significantly and consistently encoding post error/success factor. Rat A was an exception who actually was not able to solve the learning task. Only recently have some researchers started to address how trial outcomes may be encoded in single neurons in relation to future actions during learning. This study is the first time to directly capture and quantify the emerging single unit neural activity pattern in relation to the important
previous trial outcome factor. This may serve as the first step toward gaining insight on neural mechanism of learning behavior.

Disclosures: J. Si: None. W. Ma: None.

Poster

533. Executive Function: Neurophysiology

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 533.15/AA25

Topic: F.02. Animal Cognition and Behavior

Title: CPEB2 regulates hippocampus-related long-term synaptic plasticity and memory

Authors: W.-H. LU1,2,3, *C.-T. CHIEN4, Y.-S. HUANG1,2,3;

Abstract: Activity-dependent de novo protein synthesis in either axons and/or synapto-dendritic compartments is necessary to sustain long-lasting modification of synapses and consequently support long-term memory. Cytoplasmic polyadenylation element binding protein 2 (CPEB2) is a translational regulator and widely expressed in the central nervous system, so we examine whether CPEB2-controlled translation is important for learning and memory. Because of respiratory distress-associated neonatal lethality in CPEB2 knockout (KO) mice, the conditional CPEB2 wild-type (cWT) and KO (cKO) mice of which cpeb2 gene is ablated in neurons expressing Cre recombinase under the control of CaMKII promoter, were used for behavioral and electrophysiological analyses. Although CPEB2 cKO mice performed normally in anxiety-related tests, including open field and elevated plus maze, they exhibited memory consolidation deficit in hippocampus-associated Morris water maze and contextual fear conditioning tests. Moreover, certain forms of synaptic transmission, including long-term depression (LTD) and long-term potentiation (LTP), were impaired in the Schaffer collateral-CA1 region of cKO hippocampal slices. To identify the molecular and cellular defects underlying aberrant memory performances and electrophysiological responses, we used WT and KO cortical/hippocampal neurons to examine dendritic spine morphology and miniature excitatory postsynaptic current (mEPSC). CPEB2 KO neurons exhibited elongated spines and reduced mEPSC amplitude. Using the RNA-immunoprecipitation approach, we identified a list of CPEB2-bound miRNAs. In combination with literature knowledge regarding of the abnormal responses identified from the
In the aforementioned experiments, we are examining whether several candidate RNAs are dysregulated in the absence of CPEB2, thereby leading to memory defects in the cKO mice.

**Disclosures:** W. Lu: None. C. Chien: None. Y. Huang: None.

**Poster**

**534. Learning and Memory: Hippocampal Circuits**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 534.01/AA26

**Topic:** F.02. Animal Cognition and Behavior

**Support:** NIH Grant K08NS071193

**Title:** An interneuron-dependent decrease in sharp-wave ripple frequency in a mouse model of Dravet Syndrome

**Authors:** *C. S. CHEAH¹, W. A. CATTERALL¹, J. C. OAKLEY²; ¹Pharmacol., ²Neurol., Univ. of Washington, Seattle, WA

**Abstract:** Dravet syndrome (DS) is an infantile-onset epileptic encephalopathy caused by loss-of-function mutations in SCN1A. Frequent, often prolonged seizures begin at 6-9 months in a previously healthy infant, with progressive cognitive impairment and severe intellectual disability in adulthood. Seizures are pharmacoresistant and no therapy exists for cognitive impairment. Cognitive impairment may result from permanent seizure-related brain injury or, alternatively, intrinsic mutation-related changes in neuronal excitability may underlie both seizures and intellectual disability. Haploinsufficiency of Scn1a in mice (DS mice) results in a selective decrease in sodium current and reduced excitability in GABAergic interneurons, together with seizures, autistic features, and memory impairment. Mice with interneuron-specific deletion of Scn1a recapitulate the core DS phenotype, supporting impaired inhibition as the cause of seizures and co-morbidities in DS. In this work, we examined whether the reduction in interneuron excitability observed in DS mice is sufficient to impair sharp wave ripple (SWR) formation. SWRs are high-frequency hippocampal field potential oscillations (140 - 220 Hz) essential for spatial memory formation and dependent on high frequency, synchronous firing of GABAergic interneurons. We recorded SWRs bilaterally in hippocampal area CA1 with chronically implanted, fine wire electrodes in awake, behaving DS and wild-type (WT) littermates. SWRs are most prominent in non-REM sleep in both DS and WT mice, and SWR in DS mice are 26% slower than WT. Elevated body temperature increases network excitability, and the SWR rate in both WT and DS mice increased by 15% at elevated temperatures. In DS
mice this increase approximates WT SWR frequencies at normal body temperature, demonstrating that DS mice retain the capability to form faster SWRs. Parallel experiments in mice with interneuron-specific Scn1a deletion showed a similar trend, implicating inhibitory neurons as the primary effectors of the DS SWR dysfunction. Decreased SWR frequency mediated by reduced interneuron excitability, due to Scn1a haploinsufficiency, provides a potential physiologic basis for spatial memory impairment in DS mice. Similarly, reducing interneuron sodium current in a conductance-based hippocampal network model decreased SWR frequency which implies that SWR frequency is primarily determined by the intrinsic excitability of interneurons, independent of network connectivity (Unpublished work, BN Lundstrom and JC Oakley). Together, these results suggest that therapies targeted at repairing interneuron excitability may improve memory in DS.

Disclosures: C.S. Cheah: None. W.A. Catterall: None. J.C. Oakley: None.

Poster

534. Learning and Memory: Hippocampal Circuits

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 534.02/AA27

Topic: F.02. Animal Cognition and Behavior

Title: Decreased ripple frequency from low sodium conductances in an interneuron network

Authors: *B. N. LUNDSTROM*<sup>1,2</sup>, J. OAKLEY<sup>1</sup>;
<sup>1</sup>Univ. of Washington, Seattle, WA; <sup>2</sup>Mayo Clin., Rochester, MN

Abstract: Dravet Syndrome is marked by severe febrile seizures, development delays, and impaired memory (1). Mice with mutations in SCN1A, which causes Dravet syndrome, demonstrate reduced sodium currents in GABAergic interneurons (2), seizures and early death (3), and impaired memory (4). Ripples are high frequency oscillations (140-220 Hz) of field potentials generated in the hippocampus and associated with intact memory (5). Unpublished work (JC Oakley et al) has found that hippocampal CA1 ripple frequency is reduced for SCN1A heterozygote mice (mean 123 Hz, SD = 8.6 Hz, n = 4) as compared to wild-type (mean 165.5 Hz, SD 6.0 Hz, n = 4). Here the intent is to examine whether reduced sodium conductances in a network model yield reduced ripple frequencies, and to what extent reciprocal connections are required. We simulated a CA1 hippocampal network using Wang-Buzsaki conductance-based interneurons receiving Gaussian shaped excitatory stimuli. Neurons were connected by fast, strong, and shunting inhibitory synapses. Results show that ripple frequencies are reduced when sodium conductances of the inhibitory interneurons are reduced. Ripple frequency is primarily

Disclosures: B.N. Lundstrom: None. J. Oakley: None.

Poster

534. Learning and Memory: Hippocampal Circuits

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Program#/Poster#: 534.03/AA28

Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant 007051

Title: Creatine transporter deficient mice show changes in hippocampal morphology

Authors: *K. MILES, M. SKELTON;
Neurosci., Univ. of Cincinnati, Cincinnati, OH

Abstract: Creatine (Cr) is a nitrogenic acid responsible for maintaining ATP homeostasis in eukaryotic cells. The loss of Cr, through deficits in either Cr transport or synthesis, leads to significant intellectual disability, epilepsy and aphasia. The most common cause of Cr deficiency is Creatine Transporter Deficiency (CTD), is an X linked disorder that has been estimated to affect the underlying cause of 1-2 percent of the population XLID. Patients with this disorder often display intellectual disability, seizures, or deficits in memory formation. The presence of a truncated transporter disrupts creatine metabolism resulting in an overall lack of energy to carry
out cellular function. Tissues and organs with high energy demand, such as the brain and muscle, suffer most from this depletion in creatine. While the role of Cr in the cell is well understood, the mechanisms that underlie the neurologic phenotype observed in CTD patients is still unclear. The purpose of this study was to determine if the lack of Cr caused changes in brain morphology and neuronal architecture. Creatine transporter knockout (CrT-/-) mice, which show significant cognitive deficits similar to human CTD patients, were used for these studies. CrT-/- did not show any gross morphological changes in the brain compared with their CrT+/- counterparts. In order to determine if neuronal changes were present, CrT-/- mice were crossed with mice expressing eYFP driven by the promoter for the surface antigen Thy 1 (Thy1-eYFP). Neuronal density and architecture was evaluated in the hippocampus using confocal microscopy. CrT-/- mice had fewer Thy1+ neurons compared with their WT counterparts, suggesting a loss in neuronal density. Using a creatine transporter knock out (CrT KO) mouse that expresses Thy1-GFP (a cell surface antigen that depicts cellular morphology) our lab was able to image the murine hippocampus using confocal microscopy. Images revealed marked differences in neuronal density and projections between our wild type and knock out mice. Initial examination of neuronal architecture suggests that neurons from CrT-/- mice have aberrant arborization when compared to CrT+/- mice. Understanding the structural changes in these knock out animals will translate to functional anomalies within CTD in an effort to reveal more about this disease. These data suggest that the lack of brain Cr leads to abnormal hippocampal development that could be one of the underlying causes of the cognitive deficits observed in CTD. Future work will determine if the loss of Thy1+ neurons corresponds to neuronal loss and the mechanisms that lead to Cr-mediated changes in neuronal architecture.

Disclosures: K. Miles: None. M. Skelton: None.

Poster

534. Learning and Memory: Hippocampal Circuits

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 534.04/AA29

Topic: F.02. Animal Cognition and Behavior

Support: University of Connecticut Research Foundation (UCRF)
PCLB Foundation

Title: Spatial reference memory acquisition in a water maze under light and dark conditions
Authors: *S. Patel*¹, K. King², A. Dhuri², S. Lee², E. J. Markus²;
²Dept. of Psychology, Behavioral Neurosci. Div., ¹Univ. of Connecticut, Storrs, CT

Abstract: Spatial navigation requires the use of allocentric information or external distant cues in the environment. While most research has focused on spatial visual information processing, potential distal cues include other, non-visual, modalities as well. The current study compared spatial reference memory acquisition in an open water maze under light and dark conditions. Room conditions in the dark consisted of an infrared LED illuminator (850nm, <2lx), and rats wore custom fitted vests with an infrared LED (940nm) attached. Video-tracking software recorded the animals' swim latency and distance traveled until reaching a hidden platform. We examined the degree to which the light and dark groups learned the spatial reference memory task. Following asymptotic performance for the dark group, both groups were subsequently given probes under the opposite light/dark conditions to examine carry-over effects. Do rats initially trained under light conditions carry over the spatial information to the dark? And do rats trained in the dark carry over their spatial information to the light? A visible platform test was used as a control to determine the extent to which rats could see under dark conditions. In addition, a white noise control probe was used to test rat’s dependence on auditory cues in the maze room.

Disclosures: S. Patel: C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); UCRF and PCLB Foundation. K. King: C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); UCRF and PCLB Foundation. A. Dhuri: C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); UCRF and PCLB Foundation. S. Lee: C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); UCRF and PCLB Foundation. E.J. Markus: C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); UCRF and PCLB Foundation.

Poster 534. Learning and Memory: Hippocampal Circuits

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 534.05/AA30

Topic: F.02. Animal Cognition and Behavior

Support: UCRF

PCLB Foundation
Title: Inactivation of dorsal and ventral hippocampus during a temporal sequence task in a radial arm water maze

Authors: *S. LEE, A. RATHEY, D. LEW, K. KATUGAM, E. J. MARKUS; Dept. of Psychology, Univ. of Connecticut, Storrs, CT

Abstract: The hippocampus has been linked with the formation and retrieval of an experience or episodic memories. In rats, the hippocampus is divided into dorsal and ventral regions. It has been proposed that dorsal is important for spatial processing, and ventral related to fear and anxiety. However, the conflicting results from different studies suggest an interaction with task demands, and a change in sensitivity along the dorso-ventral axis. To date, most rodent studies have focused on the “what” and “where” aspects of hippocampal processing. Our study examined the “when” aspect through teaching rats a temporal sequence task in a radial arm water maze. We previously showed rats could learn seven different goal locations in sequence. In the present study, Fisher-344 rats were trained to swim to three different locations in correct sequential order. Each of the three sessions was separated by a 30 min interval, and start arms varied pseudo-randomly for each session. Muscimol, a selective GABA-A agonist, was used to temporarily inactivate hippocampal subregions in a within-animal repeated design. Different combinations of bilateral, unilateral, and contralateral infusions were used to differentiate the relative contributions of dorsal and ventral hippocampus. To what degree will hippocampal inactivation at the start of the sequence vs. mid-sequence disrupt the successful completion of the three sessions?


Poster
534. Learning and Memory: Hippocampal Circuits

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 534.06/AA31

Topic: F.02. Animal Cognition and Behavior

Support: UCRF

   PCLB Foundation

Title: Comparing dorsal and ventral hippocampus oscillations and oscillatory interaction between hippocampus and prefrontal cortex during place and response learning in rats
Authors: J. YOON\textsuperscript{1}, X. LI\textsuperscript{1}, D. KATZ\textsuperscript{1}, S. VU\textsuperscript{1}, V. WICKENHEISSE\textsuperscript{1}, A. RATHEY\textsuperscript{1}, *E. J. MARKUS\textsuperscript{2};
\textsuperscript{1}Psychology, Behav. Neurosci. Div., \textsuperscript{2}Univ. Connecticut, Storrs Manfld, CT

Abstract: It is postulated that the oscillatory dynamics in the hippocampus support cognitive function in humans and rodents. Theta (4-12 Hz) and gamma (slow: 25-55 fast: 65-90Hz), two main types of oscillation in the hippocampus, has been linked to several key cognitive operations, including spatial navigation, memory encoding and retrieval. In most studies theta and gamma oscillations were recorded from the dorsal hippocampus. However the hippocampus is not a homogenous structure, anatomical and functional dissociations exist along the dorso-ventral axis. In addition, the rodent prefrontal cortex integrates spatial information encoded in the hippocampus with mnemonic information concerning route and task rules in order to direct behavior appropriately during hippocampal dependent task. Local filed potentials (LFP) were recorded from dorsal and ventral hippocampus and from prefrontal cortex in rats while they learn “place” and “response” tasks on a plus maze. For the place task, rats were rewarded for going to the same “place” regardless of the start arm. For the response task rats were rewarded for making a right turn on the maze. These tasks differ in the degree they depend upon hippocampus. For each task, theta and gamma frequency, coherence, power and it’s correlation with running speed were compared during the decision and post reward running epoch. How theta power and coherence were modulated with learning was also analyzed, both within and across the test days. Also, band specific hippocampal-prefrontal interactions were investigated throughout the different learning stages of place and response task.

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Poster

534. Learning and Memory: Hippocampal Circuits

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 534.07/AA32

Topic: F.02. Animal Cognition and Behavior

Support: State Key Research Program of China (grant 2011CBA0043 to X-h. Z.)

Title: A distinct entorhinal cortex to dorsal hippocampal ca1 direct circuit for olfactory associative learning
Abstract: The hippocampus (HP) is a medial temporal lobe structure that critically involved in declarative memory formation and spatial navigation. The lateral- and medial-entorhinal cortex (EC) provide major inputs to HP through separated perforant pathways (PP): EC layer II -> dentate gyrus (DG) -> CA3 -> CA1, and EC layer III -> CA1, also known as tri- and di-synaptic pathway respectively. Despite of extensive knowledge about the tri-synaptic pathway, little is known about the fine-scale connectivity and function of the direct EC -> CA1 di-synaptic circuit. Here, we report that a direct circuit of lateral-EC III to distinct group of CA1 calbindin-expressing pyramidal cells (PCs) in the dorsal HP contributes to olfactory associative learning. Using light-activated channelrhodopsin (ChR2)-assistant circuit mapping, we demonstrate that medial-EC III homogeneously innervate all examined HP CA1 PCs, while lateral-EC III selectively target to the subpopulation of HP CA1 PCs that are morphologically more complex (complex PCs, cPC) and specifically expressing an intracellular Ca2+-binding protein calbindin (Calb, CB). Specific optogenetic inactivation of either Calb-expressing PCs or direct lateral-EC PP pathway in the dorsal HP CA1 region during the training severely impaired the learning process of an olfactory associating go/no-go task. Optrode recording of these identified Calb-positive cPCs showed that they exhibited and developed higher selectivity of neuronal spiking responses to odor cues over the training days compared to those Calb-negative PCs (Simple PCs, sPC). Thus, our study revealed a distinct direct circuit from the lateral-EC III to HP CA1 Calb-expressing PCs is required for the olfactory associative learning.


Poster

534. Learning and Memory: Hippocampal Circuits

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 534.08/AA33

Topic: F.02. Animal Cognition and Behavior
Support: BBRF NARSAD 23017

Title: Hippocampal-thalamo-cortical network connectivity for episodic memory and spatial processing

Authors: *N. A. KAMBI, J. M. PHILLIPS, Y. B. SAALMANN; Dept. of Psychology, Univ. of Wisconsin-Madison, Madison, WI

Abstract: Episodic memory is the explicit remembering of objects, events and the associated contexts experienced in one’s personal past. A large body of clinical and experimental work in humans has converged on the hippocampus as a critical brain region supporting episodic memory. However, the role of other regions such as the retrosplenial cortex and anterior thalamus, which have robust anatomical connections with the hippocampus, is not clear. Independently, this network has also been implicated in spatial navigation in rodents. The major aim of this study is to better understand how the hippocampal-thalamo-cortical network supports both episodic memory and spatial processing. Our working hypothesis is that the anterior thalamus plays a key role in regulating information transmission between the hippocampus and retrosplenial cortex. To measure network connectivity, we acquired high-resolution diffusion-weighted images (1 mm isotropic) from the 3T GE MR750 scanner with a 16-channel receive-only head coil in 4 anesthetized monkeys along 60 diffusion directions; (b=1000 s/mm2 and NEX=14). We used FSL for EPI distortion, eddy current and motion correction prior to Bayesian estimation of diffusion parameters, and probabilistic diffusion tractography (PDT). We used PDT to track connections between the anterior thalamus, retrosplenial cortex, and subiculum, a major output of the hippocampus. The fiber paths obtained with PDT broadly conformed to previous anatomical tracer studies. However, PDT allowed identification of projection zones within each network area specific to individual monkeys. The results showed that the subiculum connected with both medial and lateral parts of the anterior thalamus (roughly spanning all subnuclei of the anterior thalamus), whereas the retrosplenial cortex mainly connected to the lateral part of the anterior thalamus (corresponding to the approximate location of the anterior dorsal and anterior ventral subnuclei). Importantly, the retrosplenial cortex and subiculum showed partially overlapping projection zones in the lateral part of the anterior thalamus. This suggests that there is an indirect pathway between the subiculum and retrosplenial cortex via the anterior thalamus, in addition to the direct pathway. Our data suggests that the anterior thalamus is well positioned to regulate information transmission between the subiculum and retrosplenial cortex. The PDT data also enables us to target multi-contact probes to interconnected sites of the subiculum, retrosplenial cortex and anterior thalamus for simultaneous multi-site recordings during episodic-like memory recall and spatial transformations.


Poster
**Title:** Synchronization between Hippocampus and Entorhinal Cortex in behaving rats

**Authors:** *X. S. GONZALO COGNO*<sup>1,2</sup>, E. KROPFF CAUSA<sup>3,2</sup>, M. MONTEMURRO<sup>4</sup>, I. SAMENGO<sup>1,2</sup>;<sup>1</sup>Inst. Balseiro, Ctr. Atomico Bariloche, San Carlos De Bariloche, Argentina; <sup>2</sup>CONICET, Buenos Aires, Argentina; <sup>1</sup>Leloir Inst. - IIBBA, Buenos Aires, Argentina; <sup>4</sup>Fac. of Life Sciences, Univ. of Manchester, Manchester M60 1QD, United Kingdom

**Abstract:** Hippocampal place cells and entorhinal grid cells play a crucial role in spatial navigation by encoding the location of the animal in the environment. Here, we explore the degree of synchronization of the two neural populations in awake behaving rats. The animal runs along a linear track inside a bottomless car whose velocity is controlled by a computer. This setup allows us to have complete control and knowledge of all kinematical variables (position, velocity and acceleration), and to produce many repetitions of exactly the same trajectory. In these conditions, the local field potential (LFP) from both the Medial Entorhinal Cortex and the Hippocampus were recorded. We analyzed the degree of coherence between both signals. We found that the trial-to-trial variability of the phase difference between both LFPs depends on the state of motion of the animal, with increased synchronization as the rat runs faster. In this study we describe this effect quantitatively, and we explore the causal and dynamical factors through which velocity is linked to coherence.

**Disclosures:** X.S. Gonzalo Cogno: None. E. Kropff Causa: None. M. Montemurro: None. I. Samengo: None.

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**534. Learning and Memory: Hippocampal Circuits**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 534.09/AA34

**Topic:** F.02. Animal Cognition and Behavior

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Universidad Nacional de Cuyo
Consejo Nacional de Investigaciones Científicas y Técnica

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**534. Learning and Memory: Hippocampal Circuits**
Title: The influence of spatial and temporal context on oscillatory interactions between dentate gyrus, CA3, CA1, and subiculum in rats during performance of object recognition memory tasks

Authors: *J. B. TRIMPER*, C. R. GALLOWAY, K. MANDI, A. C. JONES, J. R. MANNS

Abstract: Previous studies have found that neuronal oscillations in the hippocampus relate to object recognition memory task performance in rats and monkeys. For example, during exploration of novel objects, coherence between hippocampal subregions CA3 and CA1 in rats increased markedly in the low gamma range (30 - 55 Hz) relative to a pre-exploration baseline, an increase that correlated with subsequent memory performance (Trimper et al., 2014, Hippocampus, 24, 341-353). Expanding upon these findings, we report here findings from two separate experiments asking how neuronal oscillations dynamically change throughout four of the hippocampal subregions during performance of two separate object recognition tasks designed to ask about the role of spatial and temporal context on neuronal activity. In the first task, rats responded on average to a swap in two objects’ spatial locations by significantly increasing their exploration durations relative to when the two objects were repeated in the same location. In the second task, rats explored novel objects after a repeated temporal context more than after a novel temporal context, replicating previous findings with the same task (Manns et al., in press, Animal Cognition). Initial neural analyses revealed differences in levels of coherence between hippocampal subregions during novel object encoding (exploration relative to pre-exploration baseline), with low gamma coherence increasing significantly between dentate gyrus (DG) and CA3 and between CA3 and CA1, but not between CA1 and subiculum. Significant DG-CA3, CA3-CA1, and CA1-subiculum coherence increases above 90 Hz were also observed, possibly reflecting contributions of spiking activity to the local field potentials. No coherence differences were yet observed in the theta range. Future analyses will ask how oscillatory interactions differ based on task conditions and performance on both tasks.

534. Learning and Memory: Hippocampal Circuits

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 534.11/AA36

Topic: F.02. Animal Cognition and Behavior

Support: Deutsche Forschungsgemeinschaft SFB1089

Title: Medial septal glutamatergic neurons mediate hippocampal theta oscillations and velocity correlated firing of hippocampal neurons during locomotion

Authors: *F. FUHRMANN¹, D. JUSTUS¹, H. KANEKO¹, L. SOSULINA¹, D. FRIEDRICHS¹, T. BEUTEL¹, S. SCHOCH¹, M. SCHWARZ², M. FUHRMANN², S. REMY¹,²,³;
¹Neuronal Networks, ²Neuroimmunology and Imaging Group, German Ctr. For Neurodegenerative Dis., Bonn, Germany; ³Dept. of Epileptology, Univ. of Bonn Med. Ctr., Bonn, Germany

Abstract: In the hippocampal formation the firing of principal neurons defines a cognitive map of the environment, which is used for memory-guided navigation. Precise neuronal firing at spatial locations requires the integration of locomotion velocity. It has been shown that synchronized oscillatory activity of brain circuits and neuronal activity rates have long been known to be coupled to locomotion speed. However the mechanism mediating the hippocampal state transition to higher activity levels during the initiation of locomotion remains not fully resolved. The medial septal nucleus and the diagonal band of Broca (MSDB) play a central role in the integration of locomotor programming by relaying ascending input from the pontine-hypothalamo-septal pathway to the hippocampal formation. It is well established that the medial septal nucleus is a primary regulator of theta rhythm that orchestrates the firing of hippocampal interneurons and principal neurons at theta frequencies between 5-12 Hz. We now show that glutamatergic neurons (VGluT2+) of the MSDB mechanistically link locomotion with the entrainment of theta oscillations and the speed-related regulation of hippocampal CA1 pyramidal neuron firing rates. We used cell-type specific monitoring of the Ca2+ activity of MSDB VGluT2 neurons during locomotion and optogenetically stimulated these neurons during single-cell whole-cell patch-clamp and population two-photon imaging of CA1 pyramidal neurons in awake head-fixed mice. We found locomotion-correlated activity of both MSDB VGluT2 neurons and CA1 pyramidal neurons and show that the firing frequencies of MSDB VGluT2 neurons control the initiation and velocity of locomotion as well as the entrainment of correlated theta oscillations. Combining mono-transsynaptic retrograde tracing and brain slice electrophysiology our findings reveal a disinhibitory mechanism that facilitates the synaptic integration of Schaffer collateral (SC) and perforant path (PP) input by CA1 pyramidal neurons.
during the brain-state transition from resting to locomotion. Locomotion-related firing of VGluT2 septo-hippocampal projections resulted in alveus/oriens interneuron mediated suppression of SC and PP feed-forward inhibition already before locomotion onset. Through this mechanism the velocity-dependent activity of MSDB VGluT2 neurons is translated into increased axo-somatic depolarization and higher firing rates of hippocampal CA1 pyramidal neurons during locomotion.

hypothesis, we designed a miniature microscope that is able to image calcium signals in deep brain region such as LA and distinguish subpopulations of neurons in separate colour channels. Based on previously reported miniature microscope design (Gho et al., 2011), we have created mini-microscopes that image two colour channels simultaneously and is compatible with deep brain imaging.


Poster

534. Learning and Memory: Hippocampal Circuits

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 534.13/AA38

Topic: F.02. Animal Cognition and Behavior

Support: CIHR Grant MOP-74650

Title: Linking of fear memories by temporally limited changes in both excitatory and inhibitory neuron activity in the lateral amygdala

Authors: *A. J. RASHID*1, C. YAN1, H.-L. HSIANG1, A. DECRISTOFARO1, S. PARK1, C. RAMAKRISHNAN2, K. DEISSEROTH2, P. W. FRANKLAND3, S. A. JOSSELYN3;

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Abstract: Increased excitability downstream of activation of the transcription factor CREB increases the likelihood that a neuron of the lateral amygdala (LA) will be part of a fear engram. As memory formation itself can activate CREB, subsequent formation of a distinct memory within the time window of increased excitability results in co-allocation of memories within the LA. To investigate the mechanisms by which co-allocation may occur, we used an optogenetic approach whereby viral vectors were used to co-express channelrhodopsin2 (ChR2) and red-shifted halorhodopsin (NpHR3.0) in a fraction of neurons (~20%) in the LA of mice **in vivo,** enabling neuronal excitation and inhibition by blue light and red light illumination, respectively, in the same neurons. Activation of ChR2 just prior to auditory fear conditioning resulted in selective allocation of the fear memory to opsin-expressing neurons, as indicated by the ability to reversibly inhibit fear memory expression by activation of NpHR. When mice were given a second distinct fear memory training session without ChR2 stimulation, the second memory could also be inhibited by NpHR activation, but only if the two training sessions occurred within
the time frame of increased excitability (<6h), supporting the idea that memories can be co-
allocated if formed within a limited time window. When training sessions were separated by 24h,
outside the window of increased excitability, the second memory was insensitive to NpHR
activation and thus allocated to a separate group of LA neurons. We then attempted to prevent
memory co-allocation by inhibiting neurons allocated to the first fear memory with NpHR
activation during the second training session. Surprisingly, rather than being allocated to a
distinct population of neurons, formation of the second fear memory was inhibited, suggesting
that activity associated with the first training session prevented surrounding neurons from
contributing to subsequent memory formation. This inhibition could be relieved by selectively
attenuating the activity of parvalbumin-positive interneurons in the amygdala, using the
DREADD hM4Di, concurrent with NpHR activation in principal neurons allocated to the first
memory. The resulting memory from the second training session was insensitive to NpHR
activation, confirming that each memory was allocated to separate populations in the LA.
Collectively, these results provide evidence that memory formation results in increases in both
principal neuron and inhibitory interneuron activity, transiently constraining the population of
neurons that can be involved in subsequent memory formation, thereby promoting memory co-
allocation.

Disclosures: A.J. Rashid: None. C. Yan: None. H. Hsiang: None. A. DeCristofaro: None. S.
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Josselyn: None.

Poster

534. Learning and Memory: Hippocampal Circuits

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 534.14/AA39

Topic: F.02. Animal Cognition and Behavior

Title: Destructive circuit remodeling mediates neurogenesis-induced forgetting

Authors: *A. GUSKJOLEN, J. R. EPP, L. RESTIVO, S. A. JOSSELYN, P. W.
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Abstract: The production and maturation of new neurons in the dentate gyrus of the
hippocampus provide a physical substrate for new learning1. However, computational models
also predict that the addition of new neurons should lead to degradation of previous acquired
information (i.e., forgetting)23. Consistent with this possibility, our lab recently demonstrated
that high rates of neurogenesis cause forgetting of hippocampal-dependent information learned one month earlier\textsuperscript{5}. Our current model of neurogenesis-induced forgetting is based on the idea that as newborn neurons mature and form synaptic connections, they necessarily remodel the circuitry upon which hippocampal memories are dependent. This ‘destructive remodeling’ of the circuit likely reduces the probability that a given environmental cue will reactivate the specific pattern of neural activity that mediates successful memory retrieval\textsuperscript{6}. If this model is correct, then inhibiting the synaptic integration of newborn neurons into the surrounding circuit should alleviate the forgetting phenotype. To test this hypothesis, we have developed multiple transgenic mouse lines in which the integration of new neurons is selectively disrupted. In one such line, we deleted the Rho GTPase Rac1 from neural progenitors using a cre-loxP strategy. This deletion does not affect the proliferation (Ki67\textsuperscript+ cells) or survival (DCX\textsuperscript+ cells) of newly generated neurons. However, deletion of Rac1 reduces synaptic integration as indicated by decreased dendritic growth, arborization, and mushroom spine development\textsuperscript{7}. To test the idea that forgetting depends upon integration of new neurons, we then trained these mice in a contextual fear conditioning task. Following training, half the mice were allowed free access to a running wheel for a month and the other half were housed conventionally. Whereas post-training running increased hippocampal neurogenesis and induced forgetting in control mice, conditional deletion of Rac1 prevented running-induced forgetting. Since the integration, rather than production, of new neurons is altered in the Rac1-deficient mice, these results suggest that forgetting is mediated by circuit remodeling caused by the synaptic integration of recently generated hippocampal neurons. Critically, deleting the Rac1 gene from mature CaMKII\textsuperscript+ forebrain neurons did not block the forgetting phenotype, thereby establishing the cellular specificity of this effect. Furthermore, this effect was not driven by an anxiogenic alteration, as behavior in the open field and elevated plus maze was unchanged. This set of experiments helps elucidate the mechanism underlying neurogenesis-induced forgetting.

**Disclosures:** A. Guskjolen: None. J.R. Epp: None. L. Restivo: None. S.A. Josselyn: None. P.W. Frankland: None.

**Poster**

534. Learning and Memory: Hippocampal Circuits

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 534.15/AA40

**Topic:** F.02. Animal Cognition and Behavior

**Support:** CIHR grant MOP86762
Title: Enhanced morphological development of adult generated neurons by optogenetic stimulation

Authors: *J. R. EPP*, G. VETERE, A. GUSKJOLEN, Y. NIIBORI, S. A. JOSSELYN, P. W. FRANKLAND;

1Program in Neurosciences and Mental Hlth., Hosp. For Sick Children, Toronto, ON, Canada;
2Univ. of Toronto, Toronto, ON, Canada

Abstract: Adult neurogenesis continues throughout life in the mammalian hippocampus. The lifelong addition of new neurons provides a unique form of structural plasticity that does not exist in most areas of the brain. Thousands of new neurons are produced each day although many of these do not survive. Integration of new neurons into existing circuitry has implications for hippocampal function (Akers et al.,2007) and is believed to be an activity dependent process. For example, hippocampus-dependent learning increases the survival of immature neurons during a critical time window that corresponds to the time of dendritic and axonal outgrowth (Epp et al., 2007). Furthermore, during this time window the activation of NMDA receptors on individual new neurons increases the likelihood of that neuron surviving to maturity (Tashiro et al., 2007). At the level of individual neurons it remains unclear how activity might influence the growth and development of immature neurons in the adult hippocampus. Here we asked whether lasting structural modifications of immature neurons could be produced by brief but chronic activation of new neurons. To address this question we infected dividing neurons in the dentate gyrus with a Channelrhodopsin2-GFP expressing retrovirus. We then, performed chronic daily stimulation of these neurons. Beginning at 3 days post infection we stimulated the infected neurons for 14 days. Each daily stimulation session involved 3 epochs of 1-minute stimulation spaced 3 minutes apart. The light power and frequency were 0.4 mW and 10 Hz, respectively. Twenty four hours after the final stimulation session the mice were perfused and the brains were prepared for analysis. We used CLARITY to render the brains transparent so that we could examine intact neurons without the need for sectioning and subsequent reconstruction. The labeled neurons were imaged in their entirety using confocal microscopy and were then traced semi-automatically using Imaris (bitplane). When we examined the dendritic morphology of the new neurons we found that 2 weeks of brief stimulation with light produced a robust increase in both the size and complexity of the new neurons. Stimulated neurons had greater total dendrite length and volume as well as an increase in the number of branch points and higher order branches. Additionally, at a time when spines are first beginning to appear on new neurons there was an increase in the spine density on stimulated neurons suggesting that the developmental timeline is shortened by intrinsic activity.


Poster
Hippocampal neurogenesis leads to the erasure of a cocaine conditioned place preference

Abstract: Newborn neurons are generated in the hippocampus during adulthood. Little is known however about the functional role of these new neurons and how they contribute to behaviour. Previous research proposed that ongoing neurogenesis clears established memories from the hippocampus (Frankland et al., 2013). Consistent with this, we recently found that high rates of neurogenesis induce forgetting of hippocampus-dependent spatial (water maze, Barnes maze) and fear (contextual conditioning) memories (Akers et al., 2014). These tasks were all aversively-motivated. To test whether neurogenesis-mediated forgetting generalizes to appetitively-motivated, hippocampus-dependent memories, here we used a cocaine place preference paradigm. In this task mice learn that one side of a two chamber apparatus is consistently paired with cocaine, and when later given a choice test, mice will express a preference for the cocaine paired side. We found that increasing neurogenesis (by voluntary exercise) before training did not impact the acquisition of cocaine place preference. However, increasing hippocampal neurogenesis after training weakened an established weak cocaine place preference memory. These effects were only observed when mice were conditioned using low (7.5 mg/kg) but not high (15 mg/kg), suggesting stronger memories may be resistant to neurogenesis-induced forgetting. These findings indicate that neurogenesis-mediated forgetting in the hippocampus generalizes to appetitively-motivated tasks, and provide a basis for the development of novel interventions for treating addiction and relapse.

**Abstract:** Memories are thought to be supported by a broad network of brain regions, but little is known about how such networks are organized. To address this, we previously mapped expression of the activity-regulated gene c-fos following recall of fear memory in mice across 84 brain regions. By computing inter-regional correlations we then identified brain regions that were co-activated and presumably form a network that is engaged by fear memory recall. Graph theoretical analyses of this network identified several highly-connected hub-like regions that most likely have greater overall influence on network function. Here we tested this hypothesis in two ways. First, we modeled deletion of hub and non-hub nodes on overall network function in silico. Second, we used a pharmacogenetic strategy (viral expression of the inhibitory DREADD, hM4Di) to silence neurons in different brain regions, and examine the impact of deletion of hub and non-hub regions on memory consolidation. In this latter analysis we independently targeted 25% of the network (21 brain regions). We found that “hubness” (e.g., degree centrality) predicted the degree of consolidation deficit that was observed, with silencing of the most highly-connected nodes (e.g., Reuniens thalamic nucleus) having the largest impact on fear memory consolidation ($r = 0.50, p < 0.05$). Similarly, deletion of highly-connected nodes had greatest impact on network function (e.g., largest giant component, global efficiency) in silico ($r = 0.94, p < 0.001$), and importantly predicted the deficits we observed in vivo ($r = 0.51, p < 0.05$). These results support the idea that memories are supported by distributed circuits (e.g., “mass action”) but within these broad networks some regions play more essential roles than others.

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Title: Medial prefrontal cortex parvalbumin-positive interneurons modulate spindle-ripple coupling and fear memory consolidation

Authors: *F. XIA*¹², B. A. RICHARDS³, S. A. JOSSELYN¹²⁴, K. TAKEHARA-NISHIUCHI⁴, P. W. FRANKLAND¹²⁴,
¹Hosp. For Sick Children, Toronto, ON, Canada; ²Physiol., ³Cell & Systems Biol., ⁴Psychology, Univ. of Toronto, Toronto, ON, Canada

Abstract: After a memory is initially encoded, it undergoes a period of consolidation, during which it becomes less reliant on the hippocampus (HPC) and more dependent on the medial prefrontal cortex (mPFC). Rhythmic oscillations in the mPFC, called spindles, coincide with oscillations in the HPC, called sharp-wave ripples, and this spindle-ripple coupling across brain regions is thought to facilitate memory consolidation. While fast-spiking parvalbumin-positive (PV+) interneurons fire in synchrony with spindles and ripples in the mPFC and HPC, respectively, whether they contribute to the spindle-ripple coupling, and by doing so, modulate memory consolidation, is unclear. Here, we use the designer receptor approach combined with behavior experiments and recording in freely-behaving mice, to directly manipulate the activity of PV+ interneurons in the HPC and mPFC and investigate their roles in memory consolidation. Transgenic PV::Cre mice were infused with Cre-recombinase-dependent virus carrying the designer receptor hM4Di, which allows PV+ interneurons to be silenced by the designer drug clozapine-N-oxide (CNO). After surgery, mice were trained in a contextual fear conditioning paradigm, then we silenced the PV+ interneurons. When mPFC PV+ interneurons were selectively inhibited during the consolidation period following fear conditioning, mice showed memory deficits. To explore the electrophysiological mechanisms, we performed local field potential recordings simultaneously in both the mPFC and HPC in freely-behaving mice following fear conditioning. Our preliminary data shows that fear conditioning enhanced the probability of ripple-spindle coupling. While inhibiting mPFC PV+ interneurons did not alter the density, duration or amplitude of mPFC spindles or HPC ripples, the fear learning-induced enhancement in ripple-spindle coupling was attenuated. This suggests that the mPFC PV+ interneurons may be important in coordinating the oscillations between the HPC and mPFC, and
interfering with their activity may disrupt the coupling between the two regions, and hence impair memory consolidation.

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**Poster**

**534. Learning and Memory: Hippocampal Circuits**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program/#Poster#:** 534.19/AA44

**Topic:** F.02. Animal Cognition and Behavior

**Support:** CIHR

**Title:** Restoring ability to form new, and recover old “lost”, memories in mice that model Alzheimer’s disease

**Authors:** *V. MERCALDO*¹, A. P. YIU¹, D. SARGIN¹, A. J. RASHID¹, J. CERÓN GONZÁLEZ¹, P. W. FRANKLAND¹,²,³, S. A. JOSSELYN¹,²,³; ¹Neurosci. and Mental Hlth., The Hosp. For Sick Children, Toronto, ON, Canada; ²Inst. of medical sciences, ³Dept. of physiology, Univ. of Toronto, Toronto, ON, Canada

**Abstract:** The clinical hallmark of Alzheimer's disease (AD) is a progressive decline in cognitive function. Increasing evidence indicates that b-amyloid (Ab) disrupts neurotransmission and synaptic function, possibly by promoting excessive internalization of postsynaptic AMPA-type glutamate receptors (AMPARs). These findings suggest that the memory deficits described both in patients affected by early AD and mice that model AD may be due to high levels of Ab promoting the loss of postsynaptic AMPAR. Here we tested the hypothesis that excessive AMPAR internalization could account for the memory deficits seen in mice overexpressing Ab. We found that increasing Ab either acutely or chronically in various lines of mice (TgCRND8, 5xFAD, HSV viral injection of mutated APP) disrupts both consolidation and reconsolidation of context fear conditioning and spatial memory. Preventing AMPAR endocytosis during memory encoding restored the ability of mice to form new memories (consolidation). Remarkably, preventing AMPAR endocytosis during a memory reminder enabled the recovery of an otherwise inaccessible old memory (reconsolidation) in mice that model AD. These findings elucidate the disruptive role of Ab in synaptic function and raise the possibility that restoring plasticity during memory encoding and/or retrieval by targeting the loss of postsynaptic AMPAR...
may help support the ability to form new memories as well as enable recovery of lost past memories in AD patients.


Poster

534. Learning and Memory: Hippocampal Circuits

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 534.20/AA45

Topic: F.02. Animal Cognition and Behavior

Support: Canadian Institute for Health Research Grant MOP86762

Title: Canadian Institute for Health Research Grant LT00759/2014

Authors: *J. W. KENNEY, G. VETERE, L. TRAN, Y. SOUDAGAR, S. A. JOSSELYN, P. W. FRANKLAND;

Neurosciences and Mental Hlth., The Hosp. for Sick Children, Toronto, ON, Canada

Abstract: Functional connectomes are a way of representing brain region interactions underlying a specific behavior using graph theoretical concepts. One way of determining the impact of inactivating brain regions (i.e., graph nodes) in a connectome is to remove individual network nodes and their associated edges and recalculate various global graph measures such as efficiency or size of the largest connected component. However, given the nature of brain region inter-connectivity, the effect of inactivating any individual brain region is also likely to affect the interactions of regions that are indirectly connected to the target region. In an effort to develop a more realistic estimation of the effect of inactivating a specific brain region, we have developed a simple model to propagate the effect of node inactivation beyond its immediate neighborhood. We have applied this model to a functional connectome associated with contextual fear conditioning in mice (Wheeler et al, 2013) and find that the model is able to predict the effect of inactivating various brain regions on consolidation of a contextual fear memory. We discuss the development of the model and its relationship to various other graph theoretical measures of brain region/node importance.

Title: Medial prefrontal cortex inactivation biases hippocampal network representations

Authors: *J. W. RUECKEMANN, R. J. ROBINSON, II, S. M. FOROUSHANI, R. L. LEIB, S. BOVINO, H. EICHENBAUM;
Boston Univ., Boston, MA

Abstract: In a previous study on context-guided object-reward association, we reported that inactivation of the medial prefrontal cortex (mPFC) resulted in a loss of object selectivity but did not affect spatial firing patterns in hippocampal neurons (Navawongse & Eichenbaum, J Neurosci. 2013). Here we examined the generality of these findings by characterizing the effects of mPFC inactivation on hippocampal neuronal activity in rats performing a spatial task in which temporal and spatial firing patterns prominently map paths through the maze. Neurons in CA1 of the hippocampus were extracellularly recorded during pharmacological inactivation of the mPFC as rats perform a delayed-alternation task on a T-maze. Despite profound performance deficits with prefrontal inactivation, we find place cells that selectively fire depending on the intended destination of the rat, and time cells that exhibit temporal tuning curves spanning the delay period with a reliable order. However compared to baseline recording sessions, mPFC inactivation changes the active ensemble of neurons during both running and delay epochs of the task, and alters the representations of temporally and spatially selective neurons without decreasing the information represented. Inactivation of the mPFC therefore results in a coordinated remapping of temporal and spatial representations in the hippocampal network. From these findings, we surmise that although temporal and spatial selectivity in the hippocampus is not dependent on the prefrontal cortex, the mPFC may serve to bias the network level representation of both features. These results indicate that the network driving spatial and temporal selectivity is separate from mPFC circuitry. Furthermore, we take the coordinated remapping of spatial and temporal firing properties as evidence that the two modalities share a common circuitry and are similarly modulated by functional connections with mPFC.
**Title:** Stimulation of the lateral entorhinal cortex reveals optimal frequencies for rhythmic entrainment of downstream hippocampal neurons

**Authors:** *L. M. RANGEL, K. R. KEEFE, P. D. RIVIÈRE, H. EICHENBAUM;* Boston Univ., Boston, MA

**Abstract:** Although it is known that the hippocampus plays an important role in associative memory, the circuit mechanisms that allow it to perform this function are not clearly understood. Previously, it has been shown that rhythmic coherence between the lateral entorhinal cortex (LEC) and the CA1 region of the hippocampus is tightly correlated with the onset of learning and successful performance in an associative learning task. We wished to examine whether rhythmic inputs from the LEC in a specific frequency range were sufficient to produce similar temporal coordination of single cell and local field potential activity (LFP) in downstream CA1. In urethane-anesthetized rats, we applied extracellular low intensity alternating current stimulation to superficial LEC. Using this method, we aimed to phase-bias ongoing neuronal activity at a range of different intensities and frequencies (from 1.25 - 70 Hz). Stimulation of LEC produced significant spike-phase coherence of CA1 cells to the stimulation frequency with respect to both the phase of the stimulation signal and the phase of the CA1 LFP. This spike-phase coherence was observed at some, but not all, of the frequencies used, and the spike-phase coherence was stronger with respect to the stimulation frequency than to the CA1 LFP. Together, these results suggest that communication between LEC and CA1 may occur at optimal frequency ranges, and that stimulation of LEC inputs to the hippocampus at these frequencies is sufficient to produce partial LEC-CA1 coherence.
Abstract: Hippocampal cells encode information about space, time, and other relevant dimensions of a task, and disruption of these cells' activity results in impairments in memory. In well-learned tasks or familiar environments, hippocampal representations of space typically remain stable; however certain manipulations, such as changes to an environment or new learning, cause hippocampal neurons to shift the locations of their place fields in a phenomenon known as remapping. While much is known about the coding properties and remapping of hippocampal cells on the timescale of hours to days, little is understood about the persistence of these representations and the tendency to remap over the course of weeks to months. We employed calcium imaging using a microendoscope to visualize activity of dorsal CA1 hippocampal neurons virally expressing GCamp6f in awake, behaving mice in order to investigate how large ensembles of hippocampal neurons maintain representations or remap over longer timescales. We recorded large numbers of cells as mice learned and mastered a spatial alternation task over the course of weeks. Additionally, mice performed a task where they were allowed to explore two separate arenas for several days, after which the two arenas were connected. Preliminary analysis confirms previous findings that ensemble representations of place persist over the course of weeks, but also decay, becoming more dissimilar as time progresses. We also observed evidence of remapping on both the single cell and ensemble level during learning in both tasks. Finally, we observed an overrepresentation of place fields in specific parts of an arena, and an underrepresentation in other parts. This over/under representation persisted despite global remapping of place fields during learning. Our results highlight how learning affects the organization/allocation of hippocampal neurons. Research supported by NIMH MH094263.
Title: Distinct, complementary organization of information in perirhinal cortex within a medial temporal lobe network supporting episodic memory

Authors: *C. S. KEENE, J. H. BLADON, J. R. O'KEEFE, C. D. LIU, H. EICHENBAUM; Boston Univ., Boston, MA

Abstract: Recent studies in our lab have focused on determining how the hippocampus and surrounding cortical regions interact in support of episodic memory. Analyses of single neuron activity patterns indicate the individual neurons throughout areas of the entorhinal cortex and hippocampus have mixed selectivity in representing multiple task features (spatial position, object identity, context, reward association). In contrast, representational similarity analysis (RSA) of population activity revealed that each region represents this information in a uniquely organized hierarchical manner (Mckenzie et al., Neuron 2014; Keene et al., SfN Abstracts 2014). For example, neuronal ensembles in lateral and medial entorhinal cortices encoded both object and position information. However, within medial entorhinal cortex, stimulus representations were organized primarily by position information and then by object information, whereas in lateral entorhinal cortex, stimulus representations were primarily organized by object identity and then by position information. The present study extended these findings by identifying the unique contribution of perirhinal cortex (PRC) by monitoring PRC neuronal activity in rats performing a context-guided object association task in which object-reward associations were opposite in different spatial contexts. Consistent with our previous studies, single unit analysis indicated strong representation of spatial positions within each context, object identities, the different contexts, and reward and non-reward associations. In addition, RSA revealed a
hierarchical organization of information distinct from those previously observed in the hippocampus and entorhinal cortices. PRC neuronal ensembles most strongly distinguished events of opposing reward association (rewarded vs non-rewarded), and within these reward-based schemas distinguished different object-context combinations, and then within those combinations distinguished the places in which objects appeared within each context. These findings, combined with previous observations on entorhinal and hippocampal schemas, challenge us to consider memory as the product of interactions between distinct organizational networks in the medial temporal lobe.


Poster

534. Learning and Memory: Hippocampal Circuits

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 534:25/BB2

Topic: F.02. Animal Cognition and Behavior

Support: NIMH MH052090

Title: Learning paradigm influences the organization of memory in the hippocampus

Authors: *D. J. SHEEHAN, J. W. RUECKEMANN, S. MEHROTRA, H. EICHENBAUM; Psychology, Boston Univ. Ctr. For Memory and Brain, Boston, MA

Abstract: The hippocampus is critical for the association of related events (Eichenbaum et al., 2012), and damage to this region consequently leads to a wide array of cognitive impairments (Eichenbaum & Cohen, 2014). Studies have demonstrated that the hippocampus is central to the rapid creation of relational representations within a given environment (Tse et al., 2007), which together form a schema necessary for solving a task (Preston & Eichenbaum, 2013). In the present study, we investigated the role of the hippocampus in representing the spatial relations between goal locations in a spatial discrimination task, and how hippocampal representations may support flexible utilization of stored memories. Rats implanted with drivable tetrodes in the dorsal CA1 region of the hippocampus were trained to acquire rewards on 6 arms of a 12-arm radial maze, using either go/no-go training on each 12 single arm or a set of 6 left-right discriminations between pairs of adjacent arms. After training, the flexibility of the representation of the maze was probed by requiring animals to choose between pairs of arms that had not previously been presented together. Single-arm training supported the flexibility to
choose the rewarded arm in novel probe tests, consistent with a relational schema of the entire maze including all the arm-reward associations. In contrast, rats trained on paired-arms failed the flexibility probe, consistent with separate learning of a left or right response for each individual pairwise problem. CA1 firing patterns reflected the distinction between relational and individual spatial representations of maze arms as a consequence of training procedure. At the end of single-arm training, CA1 place cells showed graded similarity in spatial coding among rewarded arms, whereas at the end of paired arm training, CA1 cells had largely non-overlapping spatial representations of the rewarded arms. Taken together, these results link relational coding in the hippocampus to schematic memory, and pattern separation in hippocampal coding to resolving competing individual representations.


Poster
534. Learning and Memory: Hippocampal Circuits
Location: Hall A
Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM
Program/#Poster#: 534.26/BB3
Topic: F.02. Animal Cognition and Behavior
Support: NIMH MH094263, MH051570, MH052090
Title: Gamma oscillations and hippocampal information flow during a context dependent learning task
Authors: *A. Johnson¹, S. McKenzie², A. Frank³, H. Eichenbaum³;
Abstract: Fast and slow frequency gamma oscillations within the hippocampus are associated with differential coupling across hippocampal inputs to CA1 (Colgin et al. 2009, Nature). CA1 and medial entorhinal cortex (mEC) are strongly coupled during fast gamma oscillations (60-140Hz) while CA1 and CA3 are strongly coupled during slow gamma oscillations (22-55Hz). Such differential coupling suggests that CA1 switches between distinct information states: a memory-driven state associated with slow gamma oscillations and a stimulus-driven state associated with fast gamma oscillations. An experiment by Colgin and colleagues show a link between gamma oscillations and differential prospective and retrospective spatial coding in a linear track task (Bieri et al. 2014, Neuron). Here we examined whether gamma oscillatory states
also guide memory in rats performing a context dependent object association task. We trained rats on the object association task in order to examine the links between gamma oscillations and memory function. We predicted that object sampling events would be associated with stronger fast gamma oscillations that emphasized mEC inputs and reduced slow gamma oscillations leading to inhibited CA3 input. We further predicted that the memory dynamics that culminated in digging behavior would produce highly similar interactions between fast and slow gamma dynamics - regardless of whether the digging behavior achieved a reward. We observed a strong reduction in the ratio of slow gamma power to fast gamma power at the onset of sampling that is consistent with a hippocampal information shift toward stimulus driven inputs from mEC. The gamma ratio quickly increased when the animal sampled from objects associated with reward consistent with a shift to memory driven inputs from CA3 that maintain an expectation of reward. The gamma ratio showed a slower increase when the animal sampled from objects associated with no reward. The reduction in the gamma ratio was less pronounced during sampling that immediately preceded digging behavior. This result was likely the product of reduced attention to current stimulus as a result of previous sampling within a trial. These results suggest that the flow of information in the hippocampus dynamically shifts during performance of a goal-directed memory task. When the animal samples an object, CA1 displays stronger fast gamma oscillations consistent with memory encoding of current stimuli and inhibited retrieval dynamics. However, when the animal samples from a rewarded object, CA1 displays a fast shift to stronger slow gamma oscillations consistent with memory retrieval and reduced stimulus input.


Poster

534. Learning and Memory: Hippocampal Circuits

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 534.27/BB4

Topic: F.02. Animal Cognition and Behavior

Support: NIMH 095297

Title: CA3 time cells

Authors: *D. M. SALZ¹, S. KHASNABISH², A. KOHLEY², B. J. KRAUS³, R. J. ROBINSON, II³, J. W. RUECKEMANN³, H. EICHENBAUM³;
Abstract: The hippocampus plays a critical role in representing the temporal organization of events that compose episodic memories. As a potential mechanism for temporal organization, several studies have identified hippocampal ‘time cells’, neurons that fire at specific moments in a fixed interval (much like ‘place cells’ fire when a rat is in specific places in a fixed space). The appearance of time cells in area CA1 is consistent with several studies that have highlighted the role of this subregion of the hippocampus in temporal processing (e.g., Kesner et al. Behav. Neurosci. 2005; Farovik et al., Learning & Memory 2010) but other subfields of the hippocampus have not been examined for temporal coding. Here we explored whether temporal processing within the hippocampus is limited to area CA1 by comparing temporal and spatial coding properties of CA1 and CA3 neurons in rats running in place on a treadmill in between alternate paths in a delayed alternation T-maze task. CA3 neurons fired at specific moments during running in place, much like CA1 time cells, and CA3 time cells are as numerous as CA1 time cells. In addition, time cells in both CA1 and CA3 were also prevalent when the memory load was eliminated by having rats run in the same path on each trial. Taken together, these results are consistent with the view that the properties of time cells parallel those of place cells in the temporal dimension, and that, like spatial processing, temporal processing is prevalent throughout the hippocampus regardless of memory demand.

Abstract: Ripples, high-frequency oscillations observed in the hippocampal formation, are believed to be associated with memory consolidation or memory replay. They are generated in the hippocampal CA3 recurrent circuits and are transmitted to its downstream networks such as the CA1 and the subiculum. The cellular mechanisms underlying their generation and transmission are, however, still poorly understood. In awake or anesthetized mice, we performed whole-cell patch-clamp recordings from membrane potentials of hippocampal neurons simultaneously with extracellular recordings of CA1 local field potentials (LFPs). After experiments, we identified the cell types and locations of recorded neurons using biocytin labeling. The LFP electrode track was found in the stratum pyramidale or radiatum of the CA1 region. CA2 neurons exhibited burst firings, which consisted of at least three spikes with an inter-spike interval of less than 10 ms. The spike bursts were not time-locked to CA1 ripples. Moreover, its membrane potentials exhibited oscillation-like fluctuations, which often emerged independently of local-field oscillations. When we measured the changes in subthreshold membrane potentials relative to the peaks of individual CA1 ripple events, we found that the CA1 neurons were hyperpolarized during about 70% of the ripple events. When the same analysis was carried out for ripples of anesthetized mice, similar hyperpolarizations were reproduced for CA2 and CA1 neurons. Therefore, a large population of hippocampal neurons are likely to be transiently suppressed during CA1 ripple events.

Disclosures: N. Matsumoto: None. Y. Ikegaya: None.

Poster

535. Learning and Memory: Modulation and Pharmacology

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 535.01/BB6

Topic: F.02. Animal Cognition and Behavior

Support: NRF-2014M3A9C4066465

Title: Ameliorating effect of erucic acid on scopolamine-induced memory impairment in mice

Authors: *E. KIM1,2, S. LEE1,2, S. JEON1,2, H. LEE1,2, H. KIM1,2, E.-R. WOO1, J. RYU1,2; 1Colleg of Pharm. Kyung Hee Univ., Seoul-City, Korea, Republic of; 2Dept. of Life and Nanopharmaceutical Sci., Kyung Hee Univ., Seoul, Korea, Republic of; 3Colleg of Pharm., Chosun Universisy, Gwangju, Korea, Republic of

Abstract: Erucic acid, a monounsaturated omega-9 fatty acid, which is contained in the seed of Raphanussativus L., is known to reduce synthesis of very long chain fatty acids (VLCFAs) and
the accumulation of the VLCFA in the brain which causes the X-linked adrenoleukodystrophy. In the present study, we investigated whether erucic acid ameliorates scopolamine-induced memory impairment using the several behavior tasks. Erucic acid (3 mg/kg, p.o.) significantly ameliorated memory impairment induced by scopolamine on the passive avoidance task, the Y-maze task and the Morris water maze task. Erucic acid also significantly enhanced cognitive performance in normal naïve mice. In addition, erucic acid significantly increased the phosphorylation levels of protein kinase C zeta (PKCζ), extracellular signal-regulated kinases (ERK) or protein kinase B (Akt) in the hippocampus. Consequently, these results suggest that erucic acid has both memory-ameliorating effects and enhancing activities, and the effects of erucic acid are partly owing to the activation of PKCζ-ERK signaling or the increase level of phosphorylated Akt in the hippocampus. Overall, erucic acid may be a potential treatment agent for cognitive deficit, such as Alzheimer’s disease (sponsored by NRF-2014M3A9C4066465).


Poster

535. Learning and Memory: Modulation and Pharmacology

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 535.02/B7

Topic: F.02. Animal Cognition and Behavior

Support: NRF Grant 2014M3A9C4066465

Title: Effects of Acanthopanax koreanum on scopolamine-induced cognitive impairment in mice

Authors: *S. LEE1,2, E. KIM1,2, H. LEE1,2, S. JEON1, H. KIM1,2, J. RYU1,2; 1Col. of Pharm. Kyung Hee Univ., Seoul-City, Korea, Republic of; 2Dept. of Life and Nanopharmaceutical Sci., Kyung Hee Univ., Seoul, Korea, Republic of

Abstract: Acanthopanax koreanum Nakai (Araliaceae) is one of widely used medicinal plants in Jeju Island, Republic of Korea, and the roots and stem bark of A. koreanum have been traditionally used as a tonic agent. In the present study, we investigated the effect of 70% etanolic extract of A. koreanum (EEAK) on scopolamine-induced memory impairment in mice using several behavioral tests such as the passive avoidance or the Y-maze tasks. Administration of EEAK (100 or 200 mg/kg, p.o.) significantly ameliorated the scopolamine-induced cognitive impairment in mice. In addition, EEAK increased the latency time during the passive avoidance task in normal naive mouse. We also observed that protein kinase B (Akt) and glycogen synthase
kinase-3β (GSK-3β) signal molecules were affected by EEAK, which means EEAK regulates cognitive function through Wnt signaling pathway. Taken together, these findings suggest that EEAK ameliorates cognitive dysfunction induced by cholinergic blockade, in part, through Akt-GSK-3β signaling pathways and would be a therapeutic potential against cognitive dysfunction such as Alzheimer’s disease (Sponsored by NRF-2014M3A9C4066465).

**Disclosures:** S. Lee: None. E. Kim: None. H. Lee: None. S. Jeon: None. H. Kim: None. J. Ryu: None.

**Poster**

535. Learning and Memory: Modulation and Pharmacology

**Location:** Hall A

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**Program#/Poster#:** 535.03/BB8

**Topic:** F.02. Animal Cognition and Behavior

**Support:** Young Researcher Fellowship 2011-2012 of Italian Ministry of Health (GR-2011-02352187)

Intramural grant Linea D3.2 – 2013

**Title:** Intramural grant Linea D3.2 – 2013

**Authors:** *S. FUSCO*1,2, A. MASTRODONATO1, M. SPINELLI1, S. COCCO1, C. RIPOLI1, S. BARBATTI1, R. PIACENTINI1, C. GRASSI1;

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**Abstract:** It is well known that early life experiences induce long-term modifications because many genes can retain a memory of exposure to the initial environment via epigenetic mechanisms. We here checked whether maternal dysmetabolic environment affects cognitive performance and key neuronal gene expression in the brain of descendants via epigenetic mechanisms. C57 adult female mice (F0) were fed with either standard or high-fat diet (SD or HFD) from 4 weeks before mating until the 3rd week of suckling. The first generation of HFD-fed mice, hereinafter referred as F1-HFD, and their descendants (F2-HFD and F3-HFD, respectively) were all fed with SD and were tested by behavioral, electrophysiological and molecular analyses. Our findings demonstrate that maternal overnutrition alters learning and memory in the offspring and next generations. All HFD-descendant mice showed a lower discrimination index than the SD mice in a standard novel object recognition paradigm (F1-HFD
These effects were associated with a significant impairment of spatial learning and memory in the Morris water maze. Time spent to reach the platform at the 3rd and the 4th training days was increased by +71.4% and +99.6%, respectively in F1-HFD; +78.1% and +91.3% in F2-HFD; +70.3% and +58.6% in F3-HFD, when compared to SD mice (n=8, p<0.01 for each group). Electrophysiological analyses on hippocampal brain slices of F1-, F2- and F3-HFD mice revealed severe deficits of long-term potentiation at CA3-CA1 synapses (ranging from -37.3% in F1-HFD to -52.2% in F3-HFD; n = 11 slices from 4 mice per group, p<0.05). Finally, maternal HFD reduced multiple BDNF transcripts and its expression at the protein level via specific epigenetic mechanisms involving BDNF regulatory sequences. More importantly, in caudal epididymis of HFD-descendants male mice we found the same epigenetic marks observed in their hippocampi. Collectively, our data suggest that maternal HFD alters BDNF expression and cognitive performances in the descendants via transgenerational epigenetic mechanisms.

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**Poster**

535. Learning and Memory: Modulation and Pharmacology

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#: 535.04/BB9**

**Topie:** F.02. Animal Cognition and Behavior

**Title:** Involvement of muscarinic acetylcholine receptors in conditional discrimination task in eyeblink conditioning in mice

**Authors:** *A. RAHMAN*\(^1\), N. TANAKA\(^1\), K. USUI\(^1,2\), S. KAWAHARA\(^1,2\);
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**Abstract:** In eyeblink conditional discrimination learning, subjects execute the conditioned response (CR) only when a preceding contextual cue is present. In the present study, we investigated the effect of muscarinic acetylcholine receptor (mACHr) antagonist scopolamine on acquisition and expression of the conditional CR in mice. We used a delay paradigm with a 350-ms tone conditioned stimulus (CS) and a 100-ms periorbital electrical shock unconditioned stimulus (US). 8-10-week-old male C57BL/6 mice were injected with saline (n = 8) or scopolamine (n = 8, 1 mg/kg, i.p.) 20 min before a random sequence of 30 CS-US paired trials
and 30 CS-alone trials with an intertrial interval of around 60-70 s. A 2-s light was delivered 3-4 s before the CS-US paired trial (cued trial) but not before the CS-alone trial (non-cued trial). During acquisition sessions, scopolamine group mice successfully developed CRs in both trials, failing to acquire the differential responses to the identical tone, while saline group mice successfully acquired the discrimination between the cued and non-cued trials. In the expression session after 7 consecutive days of acquisition sessions, administration of scopolamine to the saline group did not impair the pre-acquired discrimination as well as the expression of CR in the cued trials. Analysis of the hippocampal local field potential revealed that type 2 theta was elicited by the light cue during acquisition sessions in saline group, which was abolished by scopolamine in the subsequent expression session. These results suggested that mAChRs play an important role in acquisition but not expression of the conditional discrimination. Moreover, the hippocampal type 2 theta oscillation might not be necessary at least for expression of the pre-acquired discrimination.

**Disclosures:** A. Rahman: None. N. Tanaka: None. K. Usui: None. S. Kawahara: None.

**Poster**

535. Learning and Memory: Modulation and Pharmacology

**Location:** Hall A

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**Program#/Poster#:** 535.05/BB10

**Topic:** F.02. Animal Cognition and Behavior

**Support:** NIH Grant 1R15AG045820-01A1

**Title:** AMPAkine treatment modulates hippocampal theta power in the juvenile rat during Y-maze exploration

**Authors:** *D. G. MCHAIL, S. HUSSAIN, J. ASHRAFI, M. GREER, A. M. R. LOGHMANI, T. C. DUMAS; Dept. of Mol. Neuroscience, Krasnow Inst. for Advanced Study, George Mason Univ., Fairfax, VA

**Abstract:** Pathological development of excitatory synaptic transmission is thought to underlie many neurological disorders including epilepsy, schizophrenia, and autism spectrum disorders. A more complete understanding of these disorders is hindered in part because mechanisms that contribute to normal brain development and function remain poorly understood. We have shown that spontaneous alternation (SA) in a Y-maze emerges at the end of the third postnatal week in rats in association with changes in the subunit composition of glutamatergic AMPA receptors in
the hippocampus, a prolonged synaptic AMPA receptor response, and a reduced threshold for the induction of long-term potentiation (LTP) (Blair et al., 2013). These findings prompted interest in changes in hippocampal network oscillatory activity around three weeks of age. In adults, theta (4-12 Hz) power increases when an animal rears to investigate its environment and deliberates at a choice point. Prior work has shown that theta frequency and power increase in area CA1 across the first postnatal month. We implanted Long Evans rats at postnatal day (P) 14 with stereotrodes positioned to record neuronal activity from the cell body layer and synaptic layer of area CA1. Electrophysiological recording synchronized with video capture occurred as animals explored a transparent Y-maze for 15 minutes on P18, P19, P23, and P24. The positive allosteric modulator of AMPA receptors, CX614 (2.5 mg/kg, Cortex Pharmaceuticals), or vehicle was administered 30 min prior to each test (2 drug and 2 vehicle injections per animal). Implanted animals displayed an age-dependent increase in SA and CX614 increased SA at P18-19. CX614 increased the number of arm choice pauses and theta power during arm choice pauses at P23. The dissociation between the effects of CX614 on SA rate at P18-19 and the drug effects on theta power and arm choice pauses at P23 suggest that theta power and SA develop independently. The current findings support an age-dependent increase in theta frequency and power and implicate AMPA receptors in this process. Implanted animals alternated at lower rates than naïve control animals. Experiments testing the effects of isoflurane exposure on spontaneous alternation are underway.


Poster

535. Learning and Memory: Modulation and Pharmacology

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 535.06/BB11

Topic: F.02. Animal Cognition and Behavior

Support: CIHR37850

MOP86527

Title: Xiap regulates LTD dependent learning in mice

Authors: *J. Gibon¹, N. Unsain², K. Gamache³, R. Thomas¹, A. De Leon¹, A. Johnstone¹, P. Seguela¹, K. Nader³, P. A. Barker¹;

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Abstract: Hippocampal long-term depression (LTD) is an active form of synaptic plasticity necessary for learning involved in consolidation of spatial memory, contextual fear memory and novelty acquisition. Proteins of the apoptotic machinery are necessary components of NMDAR-dependent LTD and caspases are involved in postsynaptic remodeling and synaptic maturation. In the present study, we looked at the role of the endogenous inhibitor of caspases X-linked inhibitor of apoptosis (XIAP) in synaptic plasticity in the hippocampus. We initially investigated several behavioral tasks related to memory and interestingly, found that XIAP−/− mice show improved novelty acquisition in spatial and fear memory. Further analysis in both acute brain slices and hippocampal neurons in culture revealed that XIAP deletion results in significantly enhanced LTD, increased AMPA receptor internalization, increased caspase-3 activity and reduced synapse density. Taken together, these results indicate that basal XIAP expression regulates caspase-3 activity within synapses, thereby contributing to optimal synapse stability, synaptic plasticity and memory acquisition.


Poster

535. Learning and Memory: Modulation and Pharmacology

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 535.07/BB12

Topic: F.02. Animal Cognition and Behavior

Support: 1R01NS070009-05

Title: PGC-1α regulates transcriptional programs in a cell- and region-specific way for distinct impacts on circuit function and behavior

Authors: *L. J. MCMEEKIN1, A. S. BOHANNON2, E. W. ADLAF3, E. K. LUCAS2, L. S. OVERSTREET-WADICHE2, L. E. DOBRUNZ2, J. J. HABLITZ2, R. M. COWELL2; 1Univ. of Alabama At Birmingham, Birmingham, AL; 2Univ. of Alabama at Birmingham, Birmingham, AL; 3Icahn Sch. of Med. at Mount Sinai, New York, NY

Abstract: The transcriptional coactivator peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α) has been implicated in multiple psychiatric and neurodegenerative
diseases, however its cell-specific roles in transcriptional regulation are not wholly understood. PGC-1α is highly expressed in GABAergic interneurons, and although it plays a significant role in interneuron function, preliminary data suggests that this coactivator also plays a role in transcriptional regulation within striatal medium spiny neurons (MSNs) as well as cortical and hippocampal pyramidal neurons (PNs). To test this hypothesis, we generated mice lacking PGC-1α in these neuronal populations using cre-lox technology. Mice expressing cre-recombinase driven by the PV, EMX1, or the RGS9L promoter were crossed to those expressing loxP sites flanking exons 3-5 of the PPARG1A gene to delete PGC-1α from parvalbumin-positive (PV+) populations, PNs, and MSNs, respectively. Loss of PGC-1α within cortical PV+ interneurons leads to reductions in genes involved in synchronous neurotransmitter release (synaptotagmin-2, Syt2 and complexin-1, Cplx1), structural maintenance (neurofilament heavy chain, Nefh), and metabolism (phytanoyl-CoA hydroxylase, Phyh and isocitrate dehydrogenase 3 (NAD+) alpha, Idh3a), findings that are similarly seen in PNs of the somatosensory cortex with the exception of Cplx1 and Idh3a. Further, an additional subset of metabolic genes unchanged in cortex of these two conditional knockout lines is dramatically reduced in the hippocampus of animals lacking PGC-1α in PNs, indicating a role for this coactivator in regulating transcripts in a cell- and region-specific way. While these transcripts are reduced by a loss of PGC-1α in these two mouse lines, a loss of PGC-1α in MSNs results in an increase in these PGC-1α-dependent targets indicating a possible non-cell autonomous upregulation of these transcripts in PV+ interneurons. Reductions in PGC-1α-dependent genes translate to altered intrinsic electrophysiological properties and release of either GABA or glutamate onto post-synaptic targets demonstrating a cell-specific effect of a loss of PGC-1α on neuronal signaling and, thus, circuit function. Changes in regional circuitry are evident at the behavioral level in which a deletion of PGC-1α from these neuronal populations contribute to distinct cognitive and behavioral effects. These data shed light on how a reduction of PGC-1α function may contribute to pathophysiology of different diseases, particularly given the diverse array of cognitive and motor deficits associated with disorders in which reductions in PGC-1α have been associated.

**Topic:** F.02. Animal Cognition and Behavior

**Support:** European Research Council (NEUROSCHEMA – No 268800)

Medical Research Council (graduate studentship)

**Title:** Catecholaminergic neurons in mouse locus coeruleus are more strongly activated by novelty than catecholaminergic neurons in the ventral tegmental area

**Authors:** A. J. DUSZKIEWICZ¹, T. TAKEUCHI¹, P. SPOONER¹, K. DEISSEROTH², G. FERNÁNDEZ³, *R. G. MORRIS¹;

¹Ctr. for Cognitive and Neural Systems, The Univ. of Edinburgh, Edinburgh, United Kingdom;
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**Abstract:** RATIONALE: The ‘synaptic tagging-and-capture’ theory holds that persistence of memory traces can be altered by prior or subsequent patterns of neural activity that increase the availability of plasticity-related products (PRPs) (Frey and Morris, Nature, 1997). PRP production in the hippocampus is likely stimulated by neuromodulatory activity. Using the event arena, our laboratory has established that novel experiences can boost retention of unrelated spatial memories through activation of hippocampal (HPC) D1/D5 dopamine (DA) receptors (Takeuchi et al., Phil. Trans. R. Soc. Lond. B, 2014). Ventral tegmental area (VTA) has long been implicated as a possible source of DA in HPC (Lisman et al., TINS, 2012). Interestingly, a recent study (Smith and Greene, J. Neurosci., 2012) suggested that DA in HPC comes from axons of locus coeruleus (LC) neurons. METHODS and RESULTS: In order to identify the neuromodulatory structure(s) that may mediate the novelty effect on memory persistence, we recorded single unit activity of optogenetically identified catecholaminergic (CAergic) neurons in mouse VTA and LC in a novelty exploration paradigm. Using tyrosine hydroxylase-Cre knock-in mice and a Cre-dependent adeno-associated viral vector, we tagged CAergic neurons in VTA and LC selectively with channelrhodopsin-2 (ChR2). This enabled us to reliably identify, using an optetrode, CAergic neurons during unit recording sessions in freely moving animals. Neurons were classified as CAergic if they consistently fired spikes time-locked to 5-ms blue light pulses. We recorded activity of these optogenetically identified CAergic neurons during 5-min exploration of environments with familiar and novel floor substrates. We found that CAergic neurons in both VTA and LC selectively increased their firing rate in novel environments, relative to both a familiar environment and a home cage baseline. When normalised to their baseline firing rates, LC neurons were more strongly activated by novelty than VTA neurons, and a decline in this activation was observed as the recording environment became more familiar. CONCLUSIONS: Our results suggest that CAergic LC neurons may be more responsive to novelty than VTA neurons, and this may impact novelty-associated neuromodulatory enhancement of memory persistence, and set the stage for optogenetic activation and inactivation of these neurons during behavioural studies of memory persistence. Supported by European

Poster

535. Learning and Memory: Modulation and Pharmacology

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Program#/Poster#: 535.09/BB14

Topic: F.02. Animal Cognition and Behavior

Support: European Research Council (NEUROSCHEMA – No 268800)

Title: Catecholaminergic enhancement of initial memory consolidation in mice

Authors: *T. TAKEUCHI¹, A. J. DUSZKIEWICZ¹, M. YAMASAKI², D. TSE¹, P. SPOONER¹, M. WATANABE², K. DEISSEROTH³, G. FERNÁNDEZ⁴, R. G. M. MORRIS¹;
¹Univ. of Edinburgh, Edinburgh, United Kingdom; ²Hokkaido Univ., Hokkaido, Japan; ³Stanford Univ., Stanford, CA; ⁴Radboud Univ. Nijmegen, Nijmegen, Netherlands

Abstract: RATIONALE: The ‘synaptic tagging-and-capture’ theory of initial memory consolidation holds that memory persistence can be altered by prior or subsequent patterns of neural activity (Frey and Morris, Nature, 1997). We have developed a realistic model of everyday memory for mice and confirmed that unrelated novel experiences can facilitate the persistence of spatial memory (Takeuchi et al., Phil. Trans. R. Soc. Lond. B, 2014). Our analysis focuses on identifying the specific neuromodulatory systems that mediate this effect. An influential model points to the critical role of catecholaminergic (CAergic) neurons in the ventral tegmental area (VTA) (‘hippocampus (HPC)-VTA loop’: Lisman et al., TINS, 2011), but recent evidence also implicates locus coeruleus (LC) as a potential source of dopamine in HPC (Smith and Greene, J. Neurosci., 2012).

METHODS AND RESULTS: Tyrosine hydroxylase-Cre knock-in (Th-Cre) mice learned the win-stay rule of selectively finding, each day, the varying location of food in an event arena. Persistence of this transient spatial memory, tested 24 hour later, could be enhanced by 5-min exploration of an open field with novel floor substrates 30 min after encoding. Pharmacological blockade of dopamine D1/D5 but not β-adrenergic receptors in HPC during novelty exploration prevented the enhancement of memory persistence. Another cohort of Th-Cre mice, in which channelrhodopsin-2 (ChR2) was expressed in CAergic neurons of both VTA and LC using a Cre-dependent adeno-associated virus, was then trained on the
same task. ChR2-mediated photoactivation of CAergic neurons in LC but not in VTA 30 min after encoding, substituting for novelty, was also successful in enhancing the persistence of memory. Paradoxically, but in keeping with Smith and Greene’s results, the effect of LC photoactivation was blocked by a dopamine D1/D5 receptor antagonist in HPC. Anatomical studies using both retrograde and anterograde techniques indicate a much more prolific CAergic projection from LC to HPC than is observed from VTA. NEXT STEPS: Successfully mimicking the memory enhancing effect of post-encoding novelty by selective activation of LC CAergic neurons appears mechanistically distinct from the augmented attention at encoding through activation of CAergic neurons (Kentros et al., Neuron, 2004), but more in keeping with modified versions of the HPC-VTA loop/synaptic tagging-and-capture hypotheses. It would be valuable to know whether optogenetic silencing of LC CAergic neurons during novelty exposure limits the memory enhancing effect of novelty.


**Poster**

**535. Learning and Memory: Modulation and Pharmacology**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 535.10/BB15

**Topic:** F.02. Animal Cognition and Behavior

**Support:** ERC-2010-Ad6-268800-Neuroschema Grant

Branco Weiss - Society in Science Fellowship

**Title:** Differential consolidation induced by novelty and sleep associated with contrasting behavioural expression of hippocampal and cortical memory traces

**Authors:** *L. GENZEL, J. ROSSATO, J. JACOBSE, R. G. M. MORRIS;
Univ. of Edinburgh, Edinburgh, United Kingdom

**Abstract:** BACKGROUND: Distinct forms of memory consolidation (cellular and systems) influence the persistence of spatial memory within the hippocampus (cellular) and following hippocampal-neocortical interactions (systems). Factors influencing these processes include: (1) novelty exposure that enhances the persistence of hippocampal traces via neuromodulation; and (2) sleep that aids systems consolidation and thus cortical memory. Do such hippocampal and
cortical traces differ with respect to their association with immediate early gene (IEG) expression, behavioural expression and/or persistence over time? METHODS and RESULTS: In Expt 1, rats (n=80) were trained in watermaze (2 m diameter, 8 trials, 1 session), followed by sleep (S) or novelty + sleep deprivation (N+SD) during a post-training 6h consolidation period, and then given a probe trial 7d later. Following sacrifice, including home-cage controls, hippocampus and prefrontal cortex were extracted for qPCR analysis of gene expression. The key finding was that post-training S was associated with elevated plasticity-related IEG expression in cortex (tested at retrieval), while N+SD enhanced expression in hippocampus. In Expt 2, rats (n=96) were trained on two competing memories with training to one (8 trials, 1 session) followed by S and then the other (8 trials, 1 session) by N+SD. The escape platform was in opposite locations in the pool for the successive two sessions. Probe trials without any platform present were conducted at 7d. With this baseline protocol (n=32), the hippocampal memory was stronger than the cortical memory. However, when the rats were given pre-exposure to context cues over 3 days (n=32), the cortical memory improved selectively to the same strong level as the hippocampal memory. And when an extinction trial was scheduled at 24 hr after training (n=32), it erased the hippocampal but not the cortical memory. Conventional measures of watermaze performance were supplemented by heat-maps and cluster analysis of time spent exploring relevant regions of the pool. CONCLUSION: These data contribute to other findings suggesting a dynamic interplay of distinct types of memory consolidation that differentially affect hippocampal and cortical memory. Even in the absence of sleep, novelty boosts hippocampal memory, and is also selectively affected by a single session of extinction. In contrast, sleep is essential to stabilise cortical spatial memory, which can also be boosted by relevant prior knowledge. Cortical memory appears to be more resistant to interference and updating. Supported by ERC and Branco Weiss Society in Science


Poster
535. Learning and Memory: Modulation and Pharmacology

Location: Hall A

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CTS Award UL1TR000071

PHS Grant R25GM069285-06
Title: Adipocyte-specific over-expression of ecto-nucleotide pyrophosphate phosphodiesterase-1 leads to memory impairment in mice on a high-fat diet

Authors: *A. MILTON, J. KASPER, H. SALLAM, B. TUMURBAATAR, W.-R. ZHANG, D. TUVDENDORJ, F. LAEZZA, G. TAGLIALATELA, N. ABATE, J. HOMMEL; Univ. of Texas Med. Br., Galveston, TX

Abstract: Memory impairments typical of dementia and Alzheimer’s disease are potentiated by metabolic abnormalities such as type-2 diabetes mellitus and obesity. Obesity is a complex disease associated with of increased consumption of saturated lipids and carbohydrates combined with decreased physical activity. The disease is accompanied by symptoms of metabolic syndrome including decreased insulin sensitivity and dyslipidemia. The relationship between memory impairments and metabolic syndrome is poorly understood, highlighting the need to further explore their molecular, physiological and behavioral underpinnings. One possible link between obesity and memory impairment is Ecto-nucleotide pyrophosphate phosphodiesterase-1 (ENPP1), a transmembrane protein that negatively modulates insulin receptor activation and is over-expressed in people with insulin resistance. Over-expression of ENPP1 in adipose tissue of mice (AtENPP1-Tg) mimic the clinical symptoms of metabolic syndrome including insulin resistance, triglyceride deposition in the liver, and impaired lipid handling. The extent of CNS deficits induced by peripheral changes were studied using the AtENPP1-Tg mouse model. AtENPP1-Tg mice challenged with a high-fat diet display aberrant triglyceride and diacylglycerol content of synaptosomes in the hippocampus, a key brain region in learning and memory. To characterize the functional consequences of this aberrant lipid content, electrophysiological studies were performed on the hippocampus. We observed that CA1 pyramidal neurons of the hippocampus have severely impaired basal synaptic transmission in AtENPP1-Tg mice maintained on a high-fat diet. This is frequently observed in hippocampal circuitry disruptions and lead to memory deficits. AtENPP1-Tg mice were evaluated in the Morris Water Maze, a hippocampal-dependent model of memory in rodents. Mice given a high-fat diet spent decreased time in the goal quadrant compared to littermates on a standard (lean) diet. Additionally, AtENPP1-Tg mice on a high-fat diet spent less time in the goal quadrant compared to wild-type mice also on a high-fat diet. These findings suggest that ENPP1 potentiates memory impairments caused by consumption of a high-fat diet.


Poster

535. Learning and Memory: Modulation and Pharmacology

Location: Hall A
Title: Mice consuming a diet containing pectin fiber but not EGCG display cognitive benefits on the Morris water maze

Authors: *T. BHATTACHARYA*¹, P. PARK¹, C. RENDEIRO¹, B. D. PENCE², Y. SUN², A. J. COBERT³, K. S. SWANSON³, G. C. FAHEY³, R. W. JOHNSON³, K. W. KELLEY³, R. H. MCCUSKER³, J. A. WOODS², J. S. RHODES⁴; ¹Beckman Inst. for Advanced Sci. and Technol., ²Kinesiology and Community Hlth., ³Animal Sci., ⁴Psychology, Univ. of Illinois at Urbana-Champaign, Urbana, IL

Abstract: Dietary supplementation with (-)-epigallocatechin-3-gallate (EGCG), a flavonoid found in green tea, has been widely studied to examine its effects on cognitive function. In papers recently published by our group (Bhattacharya et al., 2015, Gibbons et al., 2014), we found no cognitive enhancements in either young or aged mice fed a diet supplemented with EGCG for 40 days. However, it is possible that longer interventions are needed in order to detect benefits in cognition. Furthermore, we hypothesize that the inclusion of a soluble dietary fiber in the EGCG diet might enhance the absorption of the flavonoid, making it more bioavailable and effective. Therefore, the goal of the present study was to investigate the impact of a diet containing EGCG and/or pectin fiber on cognitive tasks. Twenty C57BL/6J mice 6 weeks old were placed on a control (AIN-93M) or experimental diet (based upon AIN-93M) containing EGCG (1.5 mg/g) and/or pectin fiber. Pectin fiber was directly substituted in equal proportion for cellulose fiber, i.e. 50 g cellulose for 50 g pectin fiber, in the AIN-93M base. After 126 days on the control or experimental diets, animals were tested on the Morris water maze and the active avoidance tasks to measure spatial learning and memory and associative operant learning, respectively. No cognitive benefits were detected following supplementation with EGCG (p=0.46) or pectin fiber (p=0.68) on the active avoidance task. However, results indicated pectin improved spatial memory. In the 24-hour probe trial, mice given pectin fiber spent significantly more time in the target quadrant (p<0.01) and had an overall decreased average distance from the target platform (p<0.05) as compared to the other groups. Taken together, preliminary evidence suggests a pectin diet may have cognitive-enhancing properties but EGCG has not shown any effect on cognitive outcomes.

Poster

535. Learning and Memory: Modulation and Pharmacology

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 535.13/BB18

Topic: F.02. Animal Cognition and Behavior

Support: Department of Biotechnology, Govt. of India, Ministry of Science & Technology, New Delhi, India

Title: Combination strategies of environmental enrichment, physical exercise and nutritional supplementation enhance the spatial cognition and hippocampal neurogenesis in ventral subicular lesioned rats

Authors: *B. M. KUTTY*¹, V. KAPGAL², N. PREM², P. HEGDE², L. T. RAO²;
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Abstract: Subiculum is positioned between hippocampus and entorhinal cortex and is a part of the hippocampal learning system. Subiculum is involved in coding of spatial information. We have demonstrated that selective lesioning of cholinergic projections to subiculum reduced the subicular theta activity and impaired the spatial learning and memory functions in adult male Wistar rats. Similarly, chemical lesioning of ventral subiculum (VSL) by ibotenic acid resulted in hippocampal neuro-degeneration and cognitive impairment in rats. Post lesion exposure of VSL rats to enriched environment (EE) for 10 days enhanced the spatial learning performances in eight arm radial maze task but not in Morris water maze task. This suggests that functional recovery is task dependent and requires appropriate enrichment strategies. Hence, in the present study, we have developed an animal model of cognitive impairment by selective lesioning of ventral subiculum bilaterally using ibotenic acid (VSL) without hippocampal degeneration. Following lesions of ventral subiculum, the VSL rats were exposed to combination strategies (CS) of EE with voluntary wheel running along with nutritional supplementation for a period of one month. The VSL rats exposed to CS showed significant improvement in spatial navigational abilities in Morris water maze task and also showed enhanced hippocampal neurogenesis. However, the VSL rats exposed to standard housing conditions continued to show significant spatial deficits and reduced hippocampal neurogenesis. The study suggests that subiculum is important for spatial navigation and suitable enrichment strategies facilitate intrinsic plasticity events such as enhanced synaptic plasticity and adult neurogenesis leading to functional recovery. Our study supports that combination strategies of EE, physical exercise and nutritional supplements enhance the ‘cognitive reserve’ capacities of the brain. This may be of great
significance towards developing appropriate strategies to enhance functional recovery in neurodegenerative disorders such as Alzheimer’s disease.

**Disclosures:** B.M. Kutty: None. V. Kapgal: None. N. Prem: None. P. Hegde: None. L.T. Rao: None.

**Poster**

**535. Learning and Memory: Modulation and Pharmacology**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program/#/Poster#:** 535.14/BB19

**Topic:** F.02. Animal Cognition and Behavior

**Support:** NHMRC Grant APP1044887

**Title:** 17 β-estradiol regulates gamma-band oscillations in the hippocampus and related cognitive functions

**Authors:** *A. SCHROEDER;*  
Florey Dept., The Florey Inst. of Neurosci. and Mental He, Parkville, Australia

**Abstract:** Cognitive deficits in schizophrenia are functionally disabling and there is currently no treatment to address this core symptom. Schizophrenia patients exhibit reduced gamma-band oscillations (30-80Hz) during the execution of cognitive tasks. Gamma-band oscillations are generated by parvalbumin positive (PV+), fast-spiking interneurons, which are reduced in schizophrenia brains. Estrogens have beneficial effects on cognitive function and we previously showed that estradiol regulates the expression of PV+ interneurons in the hippocampus and influences related cognitive function. The current study demonstrates for the first time that estradiol regulates gamma frequency oscillations in the dorsal hippocampus and hippocampal dependent memory in female mice. We recorded brain activity from the dorsal hippocampus of female sham ovariectomized (OVX) mice, OVX mice and OVX mice with estradiol replacement, while they were in the home cage or performing Y-maze, a short-term hippocampal-dependent memory task. While gamma-band oscillations significantly increased in the control mice when these were put in a novel environment (Y-maze), this increase was absent in OVX mice and simultaneous estradiol replacement recovered this deficit. In addition, OVX mice showed a significant reduction in gamma-band frequency, specifically during decision making, which was accompanied by a significant deficit in short-term memory. Estradiol replacement rescued these deficits. Together with our previous findings, we suggest that estradiol mediates hippocampal gamma oscillations via the expression of PV+ interneurons. This study
unraveled a mechanism underlying beneficial effects of estradiol on cognitive function in schizophrenia.

Disclosures: A. Schroeder: None.

Poster

535. Learning and Memory: Modulation and Pharmacology

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 535.15/BB20

Topic: F.02. Animal Cognition and Behavior

Title: CB2 receptor agonist GP1a restores neuronal excitability and LTP alteration in epileptic rats

Authors: *A. BELMEGUENAI1,2, M. OGIER1,2, J. BODENNEC1,2, B. GEORGES1,2, L. BEZIN1,2;
1TIGER Team (Translational & Integrative Groupe In Epilepsy Research), Lyon Neurosciences Ctr. - CRNL, Bron, France; 2Inst. for Epilepsy - IDEE, Bron, France

Abstract: The effects of cannabinoids were primarily described as mediated by two types of cannabinoid receptors, CB1 receptors in the nervous system and CB2 receptors in the immune system. Recent evidence indicates that CB2 receptors are also widely expressed in the brain and are involved in cognitive processes. These observations also suggest that CB2 receptor activation may be a potential therapeutic strategy for disorders associated with different cognitive deficits. Status epilepticus (SE) is one of the most common neurologic emergencies leading to chronic epilepsy. We recently demonstrated in rats subjected to SE at weaning that hippocampal-dependent spatial memory and LTP, a candidate mechanism for learning and memory, are altered following SE (Fares et al., 2013). In this study, we observed that the transcript level of the CB2 receptor was strongly induced in the hippocampus as early as 1 day after SE, and maintained at a plateau until epilepsy onset. We then tested the hypothesis that treatment with the CB2 receptor agonist GP1a in rats subjected to SE can rescue neuronal excitability and LTP induction. Therefore, we performed whole cell patch-clamp recordings from pyramidal cells in hippocampal slices obtained from rats selected 2-3 weeks post-SE. We found that bath application of GP1a selectively enhanced the action potential firing, synaptic transmission and rescued LTP induction in a dose-dependent manner in rats subjected to SE. Altogether, our data demonstrate that molecular and cellular mechanisms underlying learning and memory can be restored in rats subjected to SE by CB2 receptor agonist GP1a treatment. If these effects are confirmed by ongoing studies using behavioural tests aimed at evaluating learning and memory.
memory, then highly specific CB2 receptor agonists may be beneficial to improve cognitive functions in patients with epilepsy.

**Disclosures:** A. Belmeguenai: None. M. Ogier: None. J. Bodennec: None. B. Georges: None. L. Bezin: None.

**Poster**

535. Learning and Memory: Modulation and Pharmacology

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 535.17/BB21

**Topic:** F.02. Animal Cognition and Behavior

**Title:** 5-HT mediated plasticity in hippocampal learning and memory

**Authors:** *C. M. TEIXEIRA, Z. ROSEN, M. HERSH, S. SIEGELBAUM, M. ANSORGE; NYSPI-Columbia Univ., New York, NY

**Abstract:** Heterosynaptic activity modulates homosynaptic strength, the central parameter underlying Hebbian learning models. While the role of serotonin in heterosynaptic modulation is well established in invertebrates, its function in the vertebrate hippocampus is not understood. Here we show that optogenetic activation of serotonergic terminals in murine CA1 is sufficient to potentiate the CA3 to CA1 synapse during low-frequency stimulation of Schaffer Collaterals. Furthermore, serotonergic fiber activity in CA1 regulates behavior, with optogenetic stimulation increasing and inhibition impairing memory expression. These data demonstrate that serotonin participates in heterosynaptic potentiation of the CA3-CA1 synapse and imply a role of this mechanism in memory formation.

**Disclosures:** C.M. Teixeira: None. Z. Rosen: None. M. Hersh: None. S. Siegelbaum: None. M. Ansorge: None.
**Topic:** F.02. Animal Cognition and Behavior

**Title:** Disappearance of the left-right asymmetry of allocation of NR2B in CA1 affects non-spatial memory in β2-microglobulin KO mice

**Authors:** *A. SHIMBO*¹, I. ITO⁴, S. WATANABE²;
¹Keio Univ., Kanagawa-Ken, Japan; ²Keio Univ., Tokyo, Japan; ³Riken, Wako, Japan; ⁴Biol., Kyusyu Univ., Fukuoka, Japan

**Abstract:** The left-right asymmetry in the brain is universal phenomena in vertebrate. Such the left-right asymmetry exists in not only a macroscopic level but also a microscopic level. In circuit-level left-right asymmetry, the allocation of NMDA receptor NR2B subunits in CA1 synapses are changing depend on which CA3 inputs come from left or right hemisphere. There are synapses which have high-density expression of NR2B (NR2B predominance synapse) and synapses which have low-density expression (non-dominant NR2B synapse). The NR2B dominance synapse has a smaller size than NR2B non-dominance synapse. The NR2B dominance synapse also changes their plasticity that long term potentiation is easy to produce. However, the impact the circuit-level left-right asymmetry on cognitive functions has not been revealed yet. Therefore, in this study, we compared the behavioral phenotypes of two strains, β2-microglobulin gene knocked out mice(β2m), in which this asymmetry disappears, and c57BL/6j mice(B6). In β2m, NR2B predominance synapse express in CA1 pyramidal cells regardless of CA3 inputs which come from left or right hemisphere, but non-dominant NR2B synapse doesn’t express. We hypnotted that β2m show deficits in hippocampal-dependent cognition because CA1 pyramidal cells in β2m may have abnormality in their synaptic plasticity due to the loss of the asymmetry of the NR2B allocation. We compared two strains in four tasks, namely, spatial cognition (Dry type Morris water maze: DWMW), working memory (8 arms radial maze task), response inhibition (Differential reinforcement of low rate: DRL), and non-spatial simple learning and extinction (straight runway task). There were no differences between two strains about DWMW and 8 radial maze task. β2m mice learned DRL and Straight runway task, though the learning speed was significantly slower than that of B6. β2m showed same average extinction speed of B6 but they didn’t show spontaneous recovery in extinction of Straight runway task. These results indicate that spatial cognition, working memory and response inhibition are normal in β2m, but they show deficits about no-spatial learning. In other words, the circuit-level left-right asymmetry is more likely to have a role in non-spatial learning. Increment of the NR2B in the CA3-CA1 synapse may occur by the disappearance of the circuit-level left-right asymmetry, hence changes CA1 pyramidal cells plasticity and their activity. It is also possible that the changing CA1 pyramidal cells activity may affect activities of other brain regions, striatum and prefrontal cortex, which are relate to non-spatial learning and spontaneous recovery.

**Disclosures:** A. Shimbo: None. I. Ito: None. S. Watanabe: None.

**Poster**
535. Learning and Memory: Modulation and Pharmacology

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 535.19/BB23

Topic: F.02. Animal Cognition and Behavior

Support: NSF Grant IOS 1146853

Title: Modulation of medial entorhinal cortex layer II principal cell circuitry by glucocorticoids

Authors: *J. HARTNER\(^1\), L. SCHRADER\(^2\,\,1\);
\(^1\)Neurosci. Program, \(^2\)Cell & Mol. Biol., 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. Stellate cells have unique firing characteristics largely due to a significant h-current as well as a complex network of inhibitory inputs. 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. In this study, we use whole-cell patch clamp electrophysiology of MEC slice preparations in mice to test synaptic changes of layer II principal cells in response to bath application of dexamethasone (1-10 µM), a synthetic glucocorticoid. We show that there is no overall effect of dexamethasone treatment on frequency of either miniature or spontaneous excitatory postsynaptic currents in MEC - LII principal cells. Interestingly, the frequency of spontaneous inhibitory postsynaptic currents (IPSCs) is significantly decreased by dexamethasone application in both principal cell types of MEC - LII. We are currently working to further characterize the functional circuitry within MEC - LII and localize the effect by investigating the effects of dexamethasone on the frequency of miniature IPSCs, within MEC - LII principal cells.

Disclosures: J. Hartner: None. L. Schrader: None.

Poster

535. Learning and Memory: Modulation and Pharmacology
Neonatal (+)-methamphetamine exposure in rats: impairments in egocentric, allocentric, working, and contextual fear memory

Authors: *S. A. JABLONSKI*1,2, A. GUTIERREZ2,3, T. M. TEE2, K. L. SUTTLING2, M. T. WILLIAMS1,2,3, C. V. VORHEES1,2,3; 1Neuro., Cincinnati Children's Hosp. Med. Ctr., Cincinnati, OH; 2Cincinnati Children's Res. Fndn., Cincinnati, OH; 3Univ. of Cincinnati, Col. of Med., Cincinnati, OH

Abstract: Neonatal treatment of rat pups with (+)-methamphetamine (MA) results in long-term egocentric learning deficits in the Cincinnati water maze (CWM) and allocentric learning deficits in the Morris water maze (MWM; e.g., Vorhees et al., 2009). The mechanism for these cognitive effects is unknown. Pretreatment with the spin-trapping agent, N-tert-butyl-α-phenylnitrone (PBN) precludes MA-induced dopamine depletion in adult rats, implicating reactive oxygen species (ROS) in adult MA neurotoxicity (Cappon et al., 1996). Here we examined which cognitive tasks are sensitive to neonatal MA and if PBN could attenuate the MA-induced deficits. Using a split-litter design, male/female pairs within each litter were treated with 10 mg/kg x 4/day MA at 2 h intervals on P6-15 or saline (Sal) with or without PBN (40 mg/kg, 30 min prior to each MA or Sal injection) creating 4 groups: Sal-Sal, PBN-Sal, Sal-MA, PBN-MA. Progeny were tested in the CWM, MWM, and radial-arm water maze (RWM) as adolescents (males) or adults (females). Male offspring were tested in a 3-day fear conditioning paradigm consisting of acquisition (day 1), contextual (day 2), and cued (day 3) conditioning. Female offspring were tested in a 2-day one-trial passive avoidance (PA) test. MA-exposed rats were impaired in the CWM, MWM, and RWM at both test ages, and MA-exposed rats were impaired in contextual, but not cued, fear conditioning. There was no main effect or interaction with PBN for any test. In the CWM, MA-treated rats showed increased errors at both ages with no changes in swim speed in a straight channel prior to CWM testing. In the MWM, MA-treated rats, regardless of age, showed impaired acquisition but no changes on probe trials and no differences in swim speed. In the RWM, adolescent male MA-treated rats showed impaired working and reference memory and adult females showed deficits in reference memory. In conditioned fear, MA-treated rats showed significantly less fear to the conditioning context compared with controls, but no cued or acquisition conditioning deficits. There were no treatment group differences during either PA acquisition or 24-h retention. These data show that neonatal MA induces multiple types of cognitive deficits, but these deficits are consistently evident in tasks
associated with hippocampal function, while sparing tests associated with other brain regions (e.g., amygdala). Additionally, maze learning deficits emerge early and persist into adulthood, but are not the result of performance effects. Finally, none of the MA-induced impairments were attenuated by PBN, suggesting that ROS do not mediate the neurobehavioral effects of neonatal MA exposure.


Poster

535. Learning and Memory: Modulation and Pharmacology

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 535.21/BB25

Topic: F.02. Animal Cognition and Behavior

Title: Post-trauma administration of the pifithrin-α oxygen analogue prevents hippocampal neuronal loss and improves cognitive deficits after experimental traumatic brain injury

Authors: *L.-Y. YANG*1,2, J.-Y. WANG2, N. GREIG3, J.-Y. WANG2;

1Grad. Inst. of Med. Sciences, Col. of Med., 2Taipei Med. Univ., Taipei City, Taiwan; 3Natl. Inst. on Aging, Natl. Inst. of Hlth., Baltimore, MD

Abstract: Traumatic brain injury (TBI) is a major cause of death and disability worldwide. Neuronal apoptosis in the hippocampus has been detected after TBI. The dysfunction of hippocampus will result in cognitive deficits in learning, memory, and spatial information processing ability. Our previous study have demonstrated that a p53 inhibitor, pifithrin-α oxygen analogue (PFT-α (O)), significantly reduced of cortical cell death and improved neurological functional outcome via anti-apoptotic mechanisms. In the present study, we examined the effect of PFT-α (O) on TBI-induced cognitive impairments. To investigate whether p53-dependent apoptosis in hippocampal neuronal loss and associated cognitive deficits, SD rats were subjected to experimental TBI followed by the administration of PFT-α or PFT-α(O) (2 mg/kg, i.v.) at 5 h after TBI. Fluoro-Jade C staining were used to stain within the hippocampal regions, including CA1 and dentate gyrus (DG) of the brain. Neurological functions including somatosensory/motor and recognition memory were assessed by behavioral tests at 24 h or 7 days post injury. PUMA , p53, 4-HNE, COX4,Annexin V and NeuN were identified by double immunofluorescence staining with cell-specific markers. Levels of mRNA encoding for p53, p53-regulated pro-apoptosis genes and caspase-3 were measured by RT-qPCR. Post-injury administration of PFT-α or PFT-α (O) at 5h significantly reduced contusion volume and improved motor outcomes at 24
h and ameliorated cognitive deficits as evaluated by novel object recognition at 7 days after TBI. PFT-α or PFT-α (O) significantly reduced (~50%) the number of FJC-positive cells in hippocampus CA1 and DG regions compared with vehicle treatment. Double immunofluorescence staining demonstrated PFT-α (O) treatment decreased and p53 and annexin V positive neurons in the hippocampal CA1 region, respectively. Furthermore, PUMA colocalized with the mitochondrial maker COX4 and the upregulation of PUMA was inhibited by PFT-α (O) after TBI. We also found that TBI-induced 4-HNE protein levels within the hippocampal CA1 neurons were significantly decreased in the PFT-α (O) treated TBI animals. Our data suggest that both PFT-α and PFT-α (O) significantly reduce hippocampal degeneration, and improve neurological and cognitive deficits in vivo via antioxidative and antiapoptotic properties.


Poster
535. Learning and Memory: Modulation and Pharmacology

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 535.22/BB26

Topic: F.02. Animal Cognition and Behavior

Title: The effects of caffeine on performance of rats in the Traveling Salesman Problem

Authors: *M. STOJANOVIC, B. CHACON, D. MUHAMMAD-MENZIES, R. BLASER; Univ. of San Diego, San Diego, CA

Abstract: The traveling salesman problem (TSP) is a combinatorial optimization problem that can be used to examine spatial cognition in human and non-human animals. Although rats appear to use a distance-minimization strategy in this task, the mechanism by which they select spatial routes in this task is not yet understood. While the TSP is similar to the radial arm maze and the Morris water maze, it may require involve different processes than other commonly used spatial tasks. Our study examined the effects of acute caffeine exposure on the route choices of rats in the TSP. Animals were given caffeine on alternating days, and tested with six different Traveling Salesman problems with and without caffeine. Caffeine interacted with task difficulty and with testing day, producing improvements only on some problems, with some measures of performance. While caffeine appears to influence behavior in the TSP, the specific processes involved will require further investigation.
Title: Reduced preference for novel social stimuli after knockout of the oxytocin receptor from the hippocampal CA2 area

1NIMH, NIH, DHHS, Bethesda, MD; 2Dept. of Oral Microbiology, Sch. of Dent., Kyungpook Natl. Univ., Daegu, Korea, Republic of

Abstract: The hippocampal area CA2 plays a functional role in various forms of social behavior. The vasopressin 1b receptor (Avpr1b) is selectively and ubiquitously expressed in CA2 pyramidal cells, and knockout of this receptor gene causes deficits in social memory and offensive aggression. We previously showed that CA2 neurons are responsive to oxytocin (Oxt) (Pagani et al., 2015) and here we show that the oxytocin receptor (Oxtr) is co-expressed with Avpr1b in nearly all CA2 pyramidal cells. We therefore wondered whether Oxtr in CA2 have similar roles as Avpr1b there. We created a Cre recombinase knockin driven by the Avpr1b promoter and crossed this line with our conditional knockout of the Oxtr (fOxtr-Avpr1bcre) in order to determine how the absence of functional Oxtr in Avpr1b-expressing neurons affects a range of social behaviors. Anxiety was measured using the elevated O-maze, Object recognition and olfactory ability were measured using a 5-trial habituation-dishabitation tests. Social recognition was measured using a similar 5-trial habituation-dishabitation test as well as a harder 2-trial test with a 30 min interval. Social novelty preference was measured in a single 5-min exposure to a littermate and a novel male in a 3-chambered cage. Offensive aggression was measured using a standard resident-intruder test. Defensive aggression was measured by placing the mouse in the home cage of a confirmed aggressive male mouse. We found that fOxtr-Avpr1bcre mice fail to exhibit a preference for investigating a novel conspecific mouse over a
littermate when presented simultaneously in a three-chamber test. These mice perform similarly to wildtypes in tests of anxiety, olfactory function, social memory and object memory, and offensive and defensive aggression. Additionally, allogrooming, social contact, and olfactory investigation were normal. We thus conclude that Oxt in the CA2 contributes to the motivation to investigate novel social stimuli. The role of Oxt signaling to the Avpr1b-expressing neurons is likely to be distinct from the role of vasopressin. This research was supported by the NIMH (Z01-MH-002498-24).


Poster

535. Learning and Memory: Modulation and Pharmacology

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 535.24/BB28

Topic: F.02. Animal Cognition and Behavior

Support: DARPA N66001-14-C-4016

NIDA grant DA023573

NIDA grant DA026487

NIDA grant DA06634

Title: Multifractal complexity of hippocampal neurons after delta-9-Tetrahydrocannabinol administration during working memory and rest

Authors: *D. FETTERHOFF*1,2, R. A. KRAFT3, R. A. Sandler4, I. OPRIS2, C. A. Sexton2, V. Z. Marmarelis3, S. A. Deadwyler2, R. E. Hampson2;


Abstract: Fractality, represented as self-similar patterns that repeat at multiple scales and resolutions, is ubiquitous in nature and the brain. Dynamic patterns of hippocampal spike trains are known to exhibit multifractal properties during working memory processing (Fetterhoff et al., Journal of Neuroscience Methods, 2015); however, it is unclear whether the multifractal properties inherent to hippocampal spike trains reflect active cognitive processing. To examine this possibility, hippocampal neuronal ensembles were recorded from rodents before, during and
after a spatial working memory task. In some test sessions, animals received tetrahydrocannabinol (THC), a component of cannabis used to impair cognitive processing. Multifractal analysis was performed on sequences of hippocampal interspike intervals to determine characteristics of monofractal long-range temporal correlations (LRTCs) - quantified by the Hurst exponent, and the degree/magnitude of multifractal complexity - quantified by the width of the singularity spectrum. Our results demonstrate that multifractal firing patterns of hippocampal spike trains are a marker of functional memory processing, as they were more complex during the working memory task and significantly reduced following administration of memory impairing THC doses. Conversely, LRTCs were largest during resting state recordings, therefore reflecting different information compared to multifractality. To investigate the heightened multifractality during the task, distributions of local Hölder exponents were extracted from sample vs. nonmatch task events and differences between drug conditions were quantified using receiver operating characteristic curves. In order to deepen conceptual understanding of multifractal complexity and LRTCs, these measures were compared to classical methods using hippocampal frequency content and firing variability measures. Results showed that LRTCs, multifractality, and theta rhythm represent independent processes, while multifractality and delta rhythm were positively correlated. Additionally, we used coefficient of variation and CV2 (Holt et al., J. Neurophys., 1996) to explain the relationship between multifractality, variability and delta rhythm. Taken together, these results provide a novel perspective on cognitive function by demonstrating that the multifractal characteristics of spike trains reflect hippocampal microcircuit processing that can be used to detect and quantify cognitive, physiological and pathological states.


Poster

535. Learning and Memory: Modulation and Pharmacology

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 535.25/BB29

Topic: F.02. Animal Cognition and Behavior

Title: The NMDA antagonist, MK-801, prevents C57BL6/J mice to orient and acquire a cognitive map in a 3D maze
**Authors:** *A. ENNACEUR*, R. M. ABUHAMDAH, D. M. HUSSAIN; P. L. CHAZOT;  
1Dept. of Pharm., 1Univ. of Sunderland, Sunderland, United Kingdom; 2Sch. of Biol. and Biomed. Sci., Univ. of Durham, Durham, United Kingdom

**Abstract:** The present study investigated whether unfamiliarity, anxiety, or deficit in encoding account for the impaired acquisition of a working memory spatial navigation task following injection of MK-801 (Dizocilpine). In a 3D maze, which is a modified version of the radial arm maze, mice are introduced directly to acquire a working memory spatial navigation task without prior habituation. The first test sessions involve anxiety but this does not prevent mice with different level of emotionality to acquire the memory test within the same number of sessions. The maze consists of 9 stems radiating from a central platform. Each stem is made of 2 segments extended from a nonagonal shaped central hub. The first segment, directly attached to the central platform, can be tilted to form an upward or downward slope and constitutes a bridge; this allows access to the second segment which is maintained level and constitutes an arm. In this maze, Balb/c mice require 4 to 5 sessions to venture onto the arms while C57/BL6J and CD-1 mice require one to two sessions, respectively. In the present experiment we used C57BL6/J mice, and the bridges of the maze were inclined upward by about 40°. Three groups of food-deprived C57BL6/J mice were trained to retrieve a food pellet from the end of each of the 9 arms in 7 consecutive sessions, one session a day. One group received saline (SAL) while a second group received MK-801 (MKD1), both for 7 sessions. A third group received saline in the first 3 sessions and MK 801 in each subsequent session (MKD4). Saline and MK-801 (0.1 mg/kg) were administered 30 min before each test session. All saline treated mice made a number of visits to the arms while MKD4 showed increased entries on the fourth day onward and MKD1 showed reduced arm entries in each session. Examination of the first 9 arm choices revealed that the number of repeated arm entries (memory errors) was significantly increased in MKD1; MKD4 were comparable to control. The present results appear to confirm that MK801 produces spatial navigation deficit in animals that are unfamiliar with the test environment. This deficit may be due to MK-801 preventing animals to establish the initial mental representation of the spatial arrangement of the maze which would enable them to orient and acquire a cognitive map. This is supported by the high number of hesitation responses demonstrated by MKD1 throughout the acquisition of the test. These hesitations are reflected by the arm/bridge ratio which reached in the last session 7/20 in MKD1 and was close to 1/1 in SAL and MKD4.

**Disclosures:** A. Ennaceur: None. R.M. Abuhamdah: None. D.M. Hussain: None. P.L. Chazot: None.

**Poster**

**535. Learning and Memory: Modulation and Pharmacology**

**Location:** Hall A
Title: Midline thalamic lesions lead to impairments on hippocampal-dependent working memory and hippocampal ACh efflux

Authors: *J. M. HALL, L. M. SAVAGE; Binghamton Univ., Binghamton, NY

Abstract: Several thalamic nuclei are involved in learning and memory, and damage to the anterior, medial and midline thalamic regions can lead to amnesia. Our previous work has shown that damage to the anterior thalamus alone impairs spatial working memory in rats as assessed by spontaneous alternation and delayed alternation (Savage et al, 2011). Furthermore, lesions to the anterior thalamus (AT) also disrupt the effectiveness of the hippocampus to mount a cholinergic response during behavior and there are strong negative correlations between behavior, AT lesion size and hippocampal acetylcholine (ACh) release. In the current experiment, we examined whether lesions to the internal medullary lamina (IML), which are known to also impair spatial memory, would also disrupt behaviorally-stimulated hippocampal ACh. We found that after IML damage spontaneous alternation behavior was impaired and the impairment was associated with reduced hippocampal ACh efflux. Furthermore, although delayed alternation was impaired on the initial day of testing, performance of IML-lesioned rats recovered during the second day of testing. These data demonstrate that similar to damage of the anterior thalamus, loss of neurons in the IML region leads to spatial memory dysfunction and this is likely due to dysregulation of the hippocampus.

Disclosures: J.M. Hall: None. L.M. Savage: None.
Title: Orexin deficiency impairs hippocampus dependent learning and memory

Authors: V. MAVANJI¹, C. M. DUFFY¹, J. P. NIXON¹, *T. A. BUTTERICK⁷,¹, C. J. BILLINGTON¹,⁶,⁵, C. M. KOTZ²,²,⁴

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Abstract: Orexin-A is an endogenous neuropeptide that regulates arousal state, sleep-wake architecture and energy homeostasis. Disruption of orexin signaling leads to sleep disturbances and increased body mass index. In addition to sleep/wake areas, orexin neurons project to medial prefrontal cortex and the hippocampus, implicating a role for orexin in cognitive function. To further understand the role of orexin in cognitive function, we characterized hippocampus-dependent learning and memory in orexin-deficient mice (orexin/ataxin-3; O/A3). We tested 1) the extent of orexin loss in O/A3 mice (immunohistochemistry); 2) whether orexin receptor 1 and 2 gene expression profiles were altered in O/A3 mice (qRT-PCR); and 3) whether orexin deficiency in O/A3 mice results in impaired acquisition and consolidation of two-way active avoidance memory, a task dependent on integrity of the dorsal hippocampus. We found that the number of orexin immunoreactive neurons was significantly reduced in 6 month old O/A3 mice (approximately 84% loss). Surprisingly, orexin receptor mRNA expression was not significantly different between control and O/A3 animals, indicating the animals could be capable of normal orexin responsiveness. The O/A3 mice showed significant impairments in TWAA task learning vs. control mice (higher response latency, p < 0.001; lower avoidances, p < 0.05; reduced total response (avoidance + escapes), p < 0.01). Relative to responses in control mice, learning impairment was also evident one week after initial training (higher response latency, p < 0.001; fewer escapes, p < 0.001; reduced total response, p < 0.001). These data highlight the usefulness of orexin/ataxin mice as a model to understand orexin mediated behaviors and neurodegenerative disorders. Further, this study demonstrates that orexin plays an important role in the consolidation of hippocampus dependent two-way active avoidance memory.


Poster

535. Learning and Memory: Modulation and Pharmacology
Title: Activation of serotonin 5-HT2A receptor delays the retrieval of spatial memory by male C57BL/6J mice in a Morris water maze task

Authors: *G. ZHANG*¹, D. CINALLI², R. W. STACKMAN, Jr²;
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Abstract: Serotonin 5-HT2A receptor (5-HT2AR) a G protein-coupled receptor distributed extensively in brain regions critical for spatial cognition, in particular the hippocampus and frontal cortex. Accumulating evidence indicates that 5-HT2AR is involved in visuospatial cognition, decision-making, executive function and hallucination. 5-HT2AR abnormalities are evident in the brains of schizophrenia patients. Serotonergic hallucinogens such as psilocybin and lysergic acid diethylamide are partial 5-HT2AR agonists. Systemic administration of 5-HT2AR antagonists impairs spatial reversal learning in an operant task. Ritanerin (a 5-HT2A/2CR antagonist) significantly reduces the escape latency and distance traveled to a hidden platform in the water maze. However, the direct influence of 5-HT2AR on the encoding, consolidation and retrieval of spatial memory remain to be determined. With a hidden-platform Morris water maze (MWM) task, we found that treatment of male C57BL/6J mice with TCB-2 (1.0 mg/kg, i.p.), a 5-HT2AR agonist, prior to or immediately after training session did not affect accurate platform search behavior on the probe test session 24 h later. Interestingly, administration of TCB-2 just prior to the MWM probe test session increased the latency of mice to locate the target quadrant without affecting the heading error to target, or the proper use of distal visual cues. To identify whether the 5-HT2AR agonist impaired visual discrimination in the MWM, a visible platform water maze was used. We found that TCB-2 did not impair the latency in which mice located the visible platform. Together, our data suggest that activation of serotonin 5-HT2A receptor delays the retrieval of spatial memory in the Morris-water maze task.

Abstract: The neurotransmitter dopamine (DA) is involved in many reward related behaviors, such as drug and alcohol addiction, as well as social learning, feeding and social interactions. With systemic drug treatments, our lab has previously found that DA D1-type receptors are involved in social learning, while DA D2-type receptors are involved in feeding behavior in the social transmission of food preferences (STFP; Choleris et al., 2011), however, the site(s) of action are unknown. The ventral tegmental area has direct dopaminergic projections to many limbic structures, including the nucleus accumbens, amygdala, and hippocampus. In particular, the hippocampus is involved in learning and memory processing, as well as social learning in the STFP in rodents. Our lab has previously found that antagonizing DA D1-type receptors in the dorsal hippocampus blocks social learning in the STFP in both male and female mice (Matta & Choleris, 2014). In the present study, we assessed the role of hippocampal DA D2-type receptors in the STFP in male and female mice. To do this, we administered the DA D2-type receptor antagonist Raclopride (at 10, 14, 18 and 20 µg/µL) into the Cornu Ammonis 1 (CA1) region of the dorsal hippocampus of adult male and female observer (OBS) mice 10 minutes prior to a 30 minute social interaction where mice had the opportunity to learn a food preference from a same-sex demonstrator (DEM) conspecific. Early results show that dorsal hippocampal infusions with the highest dose of Raclopride, at 20 µg/µL, impaired social learning in female, but not male, OBS mice. Furthermore, the social learning impairment in females could not be explained by a generalized change in feeding behavior, since total food consumption was not significantly impacted by drug treatment. These results suggest that antagonizing hippocampal DA D2-type receptors in the CA1 region blocks social learning, in a sex-specific manner. Whether the phases of the estrous cycle interacted with drug treatment to influence social learning will be discussed. This study highlights a role for hippocampal DA D2-type receptors on social learning in mice.
Abstract: The hippocampus is often referred to as a cognitive map. However, it is still not known whether and how this map is learnt and what parameters it encodes. Several lines of evidence support tuning of representation in the hippocampus by outcome-related information. Here we investigate whether local dopamine (DA) input biases hippocampal representation to a subset of available dimensions in a behaviorally relevant manner. We hypothesize that DA input from the midbrain to the hippocampus serves to mold the cognitive map to represent adaptive (reward-relevant) dimensions of the sensory input, similar to its tuning of cortico-striatal connections to behaviorally adaptive action. We recorded simultaneously from multiple single units and local field potentials in area CA1 of the dorsal hippocampus of behaving rats engaged in a navigation task in which two possible sets of cues are relevant to reward collection. The two sets of cues were manipulated in an independent manner, so as to dissociate between neuronal encoding of each dimension. Once rats had learnt to follow one set of cues (after 4-6 days), the paradigm was shifted and animals had to use the second set of stimuli in order to get reward. Our first results show that CA1 neurons can rearrange to represent the reward-relevant representation, and neuronal activity can follow the behavioral set-shift rapidly after the switch to encode the most relevant parameters. To examine the dependence of this organization on dopamine, we locally infused D1/D5 dopamine antagonists (SCH23390) to bilaterally block dopaminergic receptors in the hippocampus before the set shift. Local injection of DA antagonist slows the set shift significantly. Neuronal activity in infused rats seems to persist in encoding the previously relevant parameters for the first 2-3 days after the switch of paradigm. Then, after more training days, blocking of D1 receptors seems to lead to substantial deficits in place-cell properties. Thus, our preliminary results highlight the specific role of DA in hippocampal representations in learning, decision-making and reward.
Title: The chromatin organizer special AT-rich binding protein 2 is required for synaptic plasticity and long-term memory formation

Authors: C. REDDY¹, N. WHITTLE¹, M. KORTE⁴, N. SINGEWALD³, F. FERRAGUTI², G. DECHANT¹, *E. APOSTOLOVA¹;
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Abstract: Special AT-rich binding protein 2 (Satb2) is a homeodomain protein which binds to AT-rich DNA sequences, mediates DNA-nuclear matrix interactions and regulates transcription by modulating chromatin architecture. In the brain it is expressed mainly in the cortex and CA1 area of the hippocampus. While being crucial for establishing projection neuron identity in the embryonic CNS, its function in the adult brain remains unexplored. We hypothesized a role for Satb2 in CNS neuronal plasticity based on evidence that i) BDNF and neuronal activity upregulate Satb2 in primary hippocampal neurons and ii) mutations of SATB2 in humans have been reported to lead to severe learning and memory deficits. To study the function of Satb2 in adult CNS we generated forebrain-specific Satb2 conditional knockouts (Satb2⁵CamKII-Cre). The use of CamKII-Cre allowed for postnatal Satb2 deletion thus bypassing the effects of embryonic Satb2 inactivation on circuit formation. Satb2⁵CamKII-Cre mice developed and bred normally and did not have any gross defects of cortical and CA1 architecture up to 5 months of age. Functional analysis revealed impaired stabilization of long-term potentiation in the CA1 region of hippocampal slices from Satb2⁵CamKII-Cre mice. When subjected to contextual fear conditioning
Satb2CamKII-Cre mice showed normal fear acquisition but significantly impaired fear memory 24 h after training. They also performed poorly in other hippocampus and cortex-dependent tasks 24 h but not 1 h after training, establishing a role for Satb2 during memory consolidation. Reinstatement of Satb2 in dorsal hippocampus of Satb2CamKII-Cre mice by AAV-mediated gene delivery rescued the 24 h-fear memory deficit, further confirming the necessity of Satb2 for learning. The comparison of gene expression profiles of mutant and control CA1s in home cage and following 3 h-exploration of novel enriched environment revealed several differentially expressed genes for the genotype effect, which were further validated by qPCR. Neuroactive ligand-receptor signaling and calcium signaling pathways were enriched among the identified set of differentially expressed genes. There were no significantly regulated genes for the genotype-environment interaction indicating that Satb2 is dispensable for the early wave of transcriptional activity induced by novel environment exploration. Taken together, our behavioral, electrophysiological and gene expression data reveal a crucial role for Satb2 in the long-term adaptation of neuronal functions and long-term memory formation.

**Disclosures:** C. Reddy: None. N. Whittle: None. M. Korte: None. N. Singewald: None. F. Ferraguti: None. G. Dechant: None. G. Apostolova: None.

**Poster**

536. Learning and Memory: Genes, Signaling, and Neurogenesis I

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 536.02/BB36

**Topic:** F.02. Animal Cognition and Behavior

**Support:** NSERC

**Title:** Dissociable roles of GADD45a and GADD45b in the rat perirhinal cortex and hippocampus for object memory: Different forms of DNA methylation?

**Authors:** *K. A. MITCHNICK*¹, S. D. CREIGHTON¹, B. E. KALISCH², B. D. WINTERS¹; ¹Psychology, ²Biomed. Sci., Univ. of Guelph, Guelph, ON, Canada

**Abstract:** DNA methylation, which is catalyzed by DNA methyltransferases (DNMTs), is an epigenetic mechanism necessary for long-term memory in various brain regions, including the hippocampus (HPC). Recently we have demonstrated a necessity for DNMTs in perirhinal cortex (PRh), a structure critical for object memory, as well. More specifically, we have established a double dissociation between the necessity of de novo (DNMT3a) and maintenance (DNMT1) methyltransferases for long-term HPC- and PRh-mediated object-in-place (OiP) memory, a task
that requires object-location processing by the HPC and object identity processing by PRh. As the maintenance methyltransferase, DNMT1 is known to be involved during cell division and following DNA damage. No evidence exists for adult neurogenesis in PRh, but the DNA demethylation pathways do end in DNA damage and repair, therefore we have currently investigated the role of the growth arrest and DNA damage inducible 45 (GADD45a/b/g) family of proteins implicated in DNA demethylation, in HPC- and PRh-mediated OiP memory. Using intra-cranial administration of Accell siRNAs, the contributions of GADD45a and GADD45b were also found to be dissociable, such that only GADD45a siRNA impaired long-term OiP memory in PRh, while GADD45b siRNA impaired in the HPC. Furthermore, GADD45a and GADD45b mRNA levels were upregulated in the PRh and dentate gyrus (DG) of the HPC, respectively, following learning. GADD45b was additionally upregulated in PRh. Collectively these results demonstrate a role for GADD45b and DNMT3a in HPC-mediated object recognition, and GADD45a and DNMT1 in PRh-mediated object recognition, indicating that different epigenetic mechanisms are required for distinct mnemonic processes (spatial vs object identification) in various brain regions. As DNMT1 and GADD45a have been shown to interact and be recruited to DNA following DNA damage, we are currently conducting investigations to assess the possibility that GADD45a helps to modulate learning-induced DNA re-methylation following demethylation-induced DNA damage in PRh.


Poster

536. Learning and Memory: Genes, Signaling, and Neurogenesis I

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 536.03/BB37

Topic: F.02. Animal Cognition and Behavior

Support: NSERC OGS

Title: Dissociable roles for maintenance and de novo DNA methyltransferases in object and spatial memory in the rat perirhinal cortex and hippocampus

Authors: *S. D. CREIGHTON*¹, K. A. MITCHNICK¹, A. ALIZZI¹, B. E. KALISCH², B. D. WINTERS³;
Abstract: DNA methylation, catalyzed by DNA methyltransferases (DNMTs), is an epigenetic modification that occurs in response to environmental stimuli and elicits changes in gene expression that support cellular memory. DNA methylation is critical to many facets of long-term memory (LTM), where a dynamic balance between methylation of memory suppressing genes and demethylation of memory promoting genes is necessary. We have previously demonstrated dissociable roles for maintenance methyltransferases (DNMT1) in perirhinal cortex (PRh) and de novo methyltransferases (DNMT3a) in the hippocampus (HPC) for long-term object-in-place (OiP) memory. As the OiP task consists of object identity and spatial components, which are likely PRh- and HPC-dependent, respectively, this dissociation suggests that distinct epigenetic mechanisms might apply to different types of LTM. Here we systematically evaluated the unique contributions of DNMTs to object identity (spontaneous object recognition; SOR) and spatial (object location; OL) memory in rats. Disruption of DNMTs during SOR and OL learning by the non-selective DNMT inhibitor, RG-108, revealed a functional double dissociation: intra-PRh DNA methylation was required for long-term (24h) SOR but not OL memory, whereas intra-HPC DNA methylation was required for long-term OL memory but not SOR. Selective inhibition of PRh DNMT1, but not DNMT3a, by short-interference RNA (siRNA) impaired long-term SOR memory. Conversely, intra-HPC administration of DNMT3a siRNA, but not DNMT1 siRNA, impaired long-term OL memory. These results are consistent with differential contributions of maintenance and de novo methyltransferases to different facets of object memory processed by different brain regions. Complementary mRNA analysis will explore the expression of genes that regulate learning and memory and neurogenesis 1h following SOR and OL learning. The expression of genes identified by these experiments will also be assessed under conditions of DNMT inhibition to speak to the role of specific DNMTs in the regulation of mRNA expression. Initial results demonstrate upregulation of BDNF in PRh, but not HPC, following SOR learning, consistent with the functional role of PRh and memory promoting genes in SOR. Interestingly, following OL learning BDNF was downregulated in the dentate gyrus (DG) region of the HPC, the memory suppressing gene PP1 was downregulated in PRh, and neurogenesis genes DISC1 and Wnt were upregulated in the DG. This is the first systematic examination of unique contributions of DNMTs to different forms of memory processed in different brain regions.

Disclosures: S.D. Creighton: None. K.A. Mitchnick: None. A. Alizzi: None. B.E. Kalisch: None. B.D. Winters: None.

Poster

536. Learning and Memory: Genes, Signaling, and Neurogenesis I

Location: Hall A
Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 536.04/BB38

Topic: F.02. Animal Cognition and Behavior

Support: MRC Grant G0900177

Title: The role of histone deacetylases in object recognition memory

Authors: *A. SMITH¹, G. R. I. BARKER¹, J. B. UNEY², E. C. WARBURTON¹; ¹Sch. of Physiol. and Pharmacol., ²Sch. of Clin. Sci., Univ. of Bristol, Bristol, United Kingdom

Abstract: Acetylation of lysine residues on histone tails promotes an open chromatin conformation that favours transcription. The addition and removal of acetyl groups to and from lysine residues is dynamically regulated by the histone acetyltransferase (HAT) and histone deacetylase (HDAC) enzymes. Previous studies have shown that conditional forebrain deletion of HDACs can enhance spatial, fear and object recognition memory in mice, while HDAC overexpression impairs performance in these tasks (Guan et al 2009 Nature 459:55-60). So far, little is known about the action of HDACs in the perirhinal cortex (PRH), an area which is essential for novel object recognition memory (Barker et al 2007 J Neurosci 27(11):2948-57). In this study, adult male Lister hooded rats (n=12) were implanted bilaterally with infusion cannulas targeting the PRH. After recovery from surgery, the effect of different HDAC inhibitors (HDACis) on single item object recognition memory was tested using a novel object preference task. Infusions of either the broad spectrum HDACi trichostatin A (TSA), the HDAC1, 2 and 3-selective HDACi CI-994 or vehicle (DMSO in saline) were performed 15 minutes prior to the sample phase. During the sample phase, animals were allowed to explore two identical copies of an object for a maximum of either 40s in 240s (‘standard’ sample phase) or 20s in 120s (‘subthreshold’ sample phase). Animals were returned to the home cage for a delay of 24h before the choice phase commenced, in which animals were presented with a third copy of the sample (now familiar) object and a copy of a novel object, and allowed to explore freely for 180s. Exploration was scored by an experimenter blind to the condition of the animals. Exploration times were used to calculate a discrimination ratio (DR) using the following equation: DR = (time spent exploring novel object - time spent exploring familiar object) / (total exploration time). Rats infused with vehicle were able to remember objects when given a standard but not subthreshold sample phase. Rats infused with TSA prior to the sample phase were able to discriminate between a novel and a familiar object at test, regardless of sample phase length. However, rats infused with CI-994 were not able to discriminate between familiar and novel objects when allowed a subthreshold sample phase. Together, these results indicate that although perirhinal histone acetylation contributes to object recognition memory encoding, this contribution is not mediated by the class I HDACs 1, 2 and 3.
Title: A histone deacetylase inhibitor, trichostatin-A, induces odor preference memory extension and maintains enhanced AMPA receptor expression in the rat pup model

Authors: *S. BHATTACHARYA*, C. W. HARLEY*, J. H. MCLEAN*;  
1Bio-medical Sci., Mem. Univ. Of Newfoundland, St John's, NL, Canada; 2Dept. of Psychology, Mem. Univ. of Newfoundland, St John's, NL, Canada

Abstract: The regulation of histone deacetylase (HDAC) is thought to play a crucial role in synaptic plasticity and long term memory formation. In this study, we test the role of histone acetylation in odor preference memory in week-old rat pups following a single training trial that normally only induces 24 h memory. We predicted that trichostatin-A (TSA), an HDAC inhibitor, would promote a longer term odor preference memory. Our behavioral studies showed that intrabulbar infusion of TSA (0.05µg/µl/olfactory bulb), prior to a 10 min pairing of the conditioned stimulus (peppermint odor) with the unconditioned stimulus (intermittent stroking), prolonged odor preference memory for at least nine days in the neonate rat. Several physiological studies indicate that insertion of AMPA receptors may be responsible for increased synaptic strength in synaptic plasticity models. Increased synaptic strength is proposed to be a basic mechanism underlying natural learning and memory. Previous studies from our laboratory have shown that AMPA receptor (GluA1) membrane expression is increased at 24 h following training, at the time of odor preference memory, while 48 h later, when preference memory is no longer expressed in the one-trial model, AMPA receptor levels are similar to control levels. We hypothesized that HDAC inhibition would maintain enhanced GluA1 receptor levels in our prolonged odor preference memory model. Western blot analysis showed that GluA1 receptor membrane expression in the TSA treated group was significantly increased at 48 h in comparison with a saline + odor stroke group. Immunohistochemical data reveal a significant increase of GluA1 receptor expression (measured by optical density of staining) in the dorsolateral olfactory bulb glomeruli (TSA treated vs vehicle) five days after training. These results extend previous
evidence for a close relationship between enhanced GluA1 receptor membrane expression and memory expression. The prolonged odor preference memory was selective for the paired odor and only occurred in a specific temporal window following HDAC inhibition. The ability to compare changes in a one trial training protocol that can induce both 24 h and nine day memories through the manipulation of histone acetylation will permit tightly controlled exploration of the molecular mechanisms, and their temporal patterns, which induce and maintain shorter or longer long-term memories.

Disclosures: S. Bhattacharya: None. C.W. Harley: None. J.H. McLean: None.

Poster

536. Learning and Memory: Genes, Signaling, and Neurogenesis I

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 536.06/BB40

Topic: F.02. Animal Cognition and Behavior

Title: Glucose-induced memory enhancement is mediated by epigenetic modulation of BDNF and FGF-1 genes expression

Authors: Y. OOMURA, *T. KATAFUCHI, S. M. HOSSAIN; Dept Integr Physiol, Grad Sch. Med. Sci, Kyushu Univ., Fukuoka, Japan

Abstract: We have previously reported that intrahippocampal injection of 7 mM glucose, which is similar to the glucose concentration of the cerebrospinal fluid during food intake, facilitates spatial learning and memory in rats. The high glucose enhanced basal synaptic response and tetanus-induced long term potentiation in the rat Schaffer collateral/commissural pathway through increases in CAMKII and PKC autophosphorylations. In the present study, we further sought to clarify the cellular mechanisms of the glucose effects on memory enhancement. When glucose was increased from 3.5 mM to 7 mM, neuronal cell lines (Neuro2A) showed an increase in expression of brain-derived neurotrophic factor (BDNF) and fibroblast growth factor-1 (FGF-1), which were both known as memory related molecules, along with the enhanced phosphorylation of AKT (PKB) and CREB. In addition, the restricted glucose water drinking increased the number of dendritic spines in the mouse hippocampus. Furthermore, the glucose-induced upregulation of BDNF was blocked by the knock down of CREB using lentiviruses encoding short hairpin-RNA against CREB, while high glucose increased CREB recruitment onto the BDNF and FGF-1 promoter regions. Interestingly, glucose stimulation reduced histone deacetylase 2 (HDAC2) recruitment and increased the acetylated histones (H3K9 and H3K27) recruitment near the BDNF and FGF-1 promoter regions in the neuronal cell line and
hippocampal tissues. An HDAC inhibitor, suberanilohydroxamic acid (SAHA) increased BDNF and FGF-1 expression. These findings, taken together, suggest that glucose enhances spatial learning and memory by upregulation of BDNF and FGF-1 genes expression through the increases in phosphorylated CREB and epigenetic modulation of the genes.

Disclosures: Y. Oomura: None. T. Katafuchi: None. S.M. Hossain: None.

Poster

536. Learning and Memory: Genes, Signaling, and Neurogenesis I

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 536.07/BB41

Topic: F.02. Animal Cognition and Behavior

Support: NSF IOS-0849800 to PJC

Louisiana Board of Regents to ARH

Title: Lentiviral knock down of inhibitor-2 in the dorsal hippocampus enhances spatial memory and contextual fear conditioning

Authors: *A. PAHNG1, H. YANG2, H. XIA2, P. COLOMBO1;

Abstract: Inhibiting protein phosphatase 1 (PP1) activity has been shown to increase phosphorylation of CREB (Alberts et al., 1994; Bito et al., 1996) and enhance memory (Genoux et al., 2002). Inhibitor-2 (I-2) is a protein that associates with the catalytic domain of PP1 and can modify the activity of PP1. Early on, researchers reported that the I2 protein inhibits protein phosphatase activity in vitro (Brandt et al., 1975; Huang and Glinsmann, 1976), whereas more recent experiments with yeast homologs showed that I2 both activates (Tung et al., 1995; Nigavekar et al., 2002) and inhibits PP1 (Tung et al., 1995). A lentiviral construct expressing genomic shRNA that corresponds to the 3’ untranslated region of I-2 mRNA knocks down I-2 expression and decreases PP1 activity (Hou et al., 2013). In the current experiment, we tested the hypothesis that localized gene silencing of I-2 in the dorsal hippocampus enhances memory for various hippocampus-dependent tasks in rats and increases levels of phosphorylation of CREB in the dorsal hippocampus due to inhibition of PP1. In comparisons with controls, rats infused with I2-shRNA demonstrated enhanced spatial memory and contextual fear conditioning. In addition, lenti-I2-shRNA infusion significantly increased levels of pCREB in the dorsal hippocampus. Our
results support the hypothesis that knocking down I-2 in the hippocampus enhances memory for aversively motivated hippocampus-dependent tasks and increases phosphorylation of CREB, most likely by down-regulating PP1 activity. These data suggest that I-2 is a novel suppressor of memory.

**Disclosures:** A. Pahng: None. H. Yang: None. H. Xia: None. P. Colombo: None.

**Poster**

536. Learning and Memory: Genes, Signaling, and Neurogenesis I

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#Poster#:** 536.08/BB42

**Topic:** F.02. Animal Cognition and Behavior

**Support:** NIH Grant MH086499

NIH Grant MH083496

Hope for Depression Research Foundation

Brain and Behavior Research Foundation

**Title:** VGF and its C-terminal peptide TLQP-62 regulate memory formation in the hippocampus via a BDNF-TrkB-dependent mechanism

**Authors:** *W.-J. LIN¹, C. JIANG¹, M. SADAHIRO¹, O. BOZDAGI², L. VULCHANOVA³, C. M. ALBERINI⁴, S. R. SALTON⁴, ⁵

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**Abstract:** Regulated expression and secretion of brain-derived neurotrophic factor (BDNF), which activates TrkB receptor signaling, is known to play a critical role in cognition. Identification of additional modulators of cognitive behavior that regulate activity-dependent BDNF secretion and/or potentiate TrkB receptor signaling, would therefore be of considerable interest. In this study we show that in the adult hippocampus, expression of the granin family gene Vgf and secretion of its C-terminal VGF-derived peptide TLQP-62 are required for fear memory formation. We found that hippocampal VGF expression and TLQP-62 levels were transiently induced after fear memory training, and that sequestering secreted TLQP-62 peptide in the hippocampus immediately after training impaired memory formation. Reduced VGF
expression was found to impair learning-evoked Rac1 induction and phosphorylation of synaptic plasticity markers cofilin and synapsin in the adult hippocampus. Moreover, TLQP-62 induced acute, transient activation of the TrkB receptor and subsequent CREB phosphorylation in hippocampal slice preparations, and its administration immediately after training enhanced long-term memory formation. A critical role of BDNF-TrkB signaling as a downstream effector in VGF/TLQP-62-mediated memory consolidation was further revealed by post-training activation of BDNF-TrkB signaling, which rescued impaired fear memory resulting from hippocampal administration of anti-VGF antibodies or germline VGF ablation. We propose that VGF is a critical component of a positive BDNF-TrkB regulatory loop, and upon its induced expression by memory training, the TLQP-62 peptide rapidly reinforces BDNF-TrkB signaling, regulating hippocampal memory consolidation.


Poster

536. Learning and Memory: Genes, Signaling, and Neurogenesis I

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 536.09/BB43

Topic: F.02. Animal Cognition and Behavior

Support: JSPS grant, Japan

Title: The special phospholipids, plasmalogens, enhance memory via increasing BDNF and other memory related gene expression in murine hippocampus

Authors: *M. HOSSAIN¹, T. KATAFUCHI¹, K. MIAKE²;
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Abstract: The special phospholipids, Plasmalogens (Pls), are found to be reduced in the brain of Alzheimer’s disease (AD) patients, but the precise meaning behind this reduction was unknown. Hippocampus, which plays a role in the formation of memory, is enriched in ethanolamine Pls (PlsEth). To elucidate the possible involvement of Pls in the hippocampus-dependent memory, we have knockdown the GNPAT (glyceronephosphate O-acyltransferase), a Pls synthesizing enzyme, by intra-hippocampal injection of shRNAs. A significant knockdown of GNPAT expression and a reduction of Pls in the hippocampus were observed in those mice after 1 week to 5th week following the shGNPATs injection. Morris water maze test showed a significant
reduction of the spatial memory among the mice of reduced Pls group, suggesting that hippocampal Pls are necessary for the memory formation. We found a reduction of BDNF expression as well as other memory related gene expression in the shGNPATs injected hippocampus. Interestingly, Pls-diet for 6 weeks in C57Bl/6 adult male mice significantly increased the spatial memory associated with the increase BDNF and other memory related gene expression. Increased activation of AKT and ERK along with the enhanced recruitment of CREB onto the BDNF promoters were found in the hippocampus. To the detail mechanism, we also found that Pls are enriched in the lipid rafts and increases the TrkB contents in these rafts to enhance BDNF signaling. Pls-diet also enhanced the synaptic activity associated with the increased LTP (CA1-CA3), number of dendritic spines of the hippocampal neuron. In addition, we also found that Pls-diet mediated memory enhancement was cancelled by shBDNF and shTrkB injection to the hippocampus. Our present study confirms for the first time that Pls in the hippocampus are very important to maintain our memory and do so via enhancing the BDNF signaling through the increasing of TrkB into the lipid rafts.

**Disclosures:** M. Hossain: None. T. Katafuchi: None. K. Mika: None.
intra-trial interval did not reveal a significant difference between ZC3H14 KO and WT mice (P = 0.77), post-hoc analysis of NOR videos with Noldus EthoVision XT 10.0 software showed a phenotype of hyperactivity in knockout mice as measured by total distance traveled (P = 0.001). This phenotype could be the result of deficient cortico-amygdala processing leading to a lack of healthy fearful behavior in the mice, as seen by a significant increase in frequency of entry into the center of the arena in ZC3H14 KO mice (P = 0.0047) compared to control mice. A 12-day water radial arm maze (WRAM) procedure indicated a trend toward cognitive dysfunction in ZC3H14 KO mice as measured by total latency to find platforms (P = 0.16) and a significant impairment of working memory (P = 0.035) when measured over the nine days it took each genotype to reach a sufficient level of performance on the WRAM so as to indicate having fully learned the task (Figure). Taken together, these results signify a quantifiable phenotype of cognitive dysfunction in ZC3H14 KO mice. These mice can be exploited to explore the critical function of the ZC3H14 RNA binding protein in the CNS.


Poster
536. Learning and Memory: Genes, Signaling, and Neurogenesis I
Location: Hall A
Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM
Program/#Poster#: 536.11/BB45
Topic: F.02. Animal Cognition and Behavior
Support: Shota Rustaveli National Science Foundation, Georgia
Title: Proteomic studies of plasma membrane-mitochondrial proteins involved in recognition memory of visual imprinting in chicks

Authors: *R. O. SOLOMONIA*¹², M. MEPARISHVILI¹, G. MARGVELANI¹, M. NOZADZE¹², E. MIKAUTADZE², T. KIGURADZE², B. J. MCCABE³;
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Abstract: Visual imprinting is a learning process by which young animals come to recognize a visual stimulus by being exposed to it (training) and consequently approach it in preference to other stimuli. Converging evidence implicates the intermediate and medial mesopallium (IMM) of the domestic chick forebrain in memory for a visual imprinting stimulus [1]. A number of learning-related, time-dependent changes have been identified in the IMM after training. The results indicate progression from transient to trophic synaptic modification, culminating in stable recognition memory. Several such changes have been found in plasma membrane and mitochondrial proteins [2]. We undertook a proteomic investigation of the plasma membrane-mitochondrial P2 fraction to identify proteins involved in visual imprinting. Two-dimensional gel electrophoresis with subsequent mass spectrometry was employed to identify differentially expressed proteins across chicks with different estimated levels of imprinting 24 h after training. We further inquired whether the amounts of those proteins in the IMM and a control region (posterior pole of the nidopallium, PPN) are correlated with memory for the imprinting stimulus. Memory strength was positively correlated with the amounts of the following proteins in the left IMM: (i) membrane cognin; (ii) a protein resembling the P32 subunit of splicing factor SF2; (iii) voltage dependent anionic channel; (iv) dynamin-1; (v) heterogeneous nuclear ribonucleoproteins A2/B1. Training significantly increased levels of (i)-(iii), suggesting that these proteins change as a consequence of learning. The possibility arises that the changes described are mediated by an increase in the number of mitochondria. However, the copy number of mitochondrial DNA did not change significantly. Of three transcription factors involved in mitochondrial biogenesis, the level of peroxisome proliferator-activated receptor gamma coactivator 1-alpha did not show significant changes, whereas the amounts of mitochondrial transcription factor-A and nuclear respiratory factor 1 increased significantly with the strength of learning. The issue of mitochondrial number is thus as yet unresolved. The results identify membrane and mitochondrial proteins that may contribute to memory and are consistent with the hypothesis [2] that mitochondrial proteins in the IMM exhibit a left-biased specialization with respect to imprinting memory.


Title: The involvement of MSK1 in experience-dependent remodelling of hippocampal synaptic plasticity

Authors: *L. PRIVITERA¹, L. MORE¹, J. S. ARTHUR², B. G. FRENGUELLI¹;
¹Sch. of Life Sci., Univ. of Warwick, Coventry, United Kingdom; ²Col. of Life Sci., Univ. of Dundee, Dundee, United Kingdom

Abstract: An enriched environment (EE) provides additional sensory, motor, cognitive and social stimulation over and above that provided by standard housing conditions (SH) for experimental animals. EE strongly influences the connectivity and development of the brain and causes profound changes in neuronal structure and function. The molecular mechanisms underlying the effects of EE are not fully understood, but the involvement of the neurotrophin BDNF has been proposed as one of the key factors in converting sensory experience into enduring changes at the cellular and behavioural level. Mitogen- and stress-activated protein kinase 1 (MSK1) is activated following stimulation of TrkB receptors by BDNF. MSK1 subsequently regulates gene transcription via phosphorylation of its downstream targets CREB and histone H3. We have previously demonstrated that mice carrying an inactivating kinase-dead (KD) knock-in point mutation of the MSK1 gene failed to show enhancement of synaptic transmission in response to EE and showed a blunted increase in dendritic spines [1]. This suggests that MSK1 is a key regulator of experience-dependent synaptic adaptation, an action that likely revolves around its ability to directly influence transcription. Here, we explored whether EE improved hippocampus-dependent basal synaptic transmission and plasticity in an MSK1-dependent manner. To this end, we reared wild-type (WT) and MSK1 KD mice in SH or in EE from birth to 2.5-5 months of age. MSK1 KD mice displayed smaller fEPSPs compared to WT mice but had similar presynaptic fiber volley amplitudes and paired-pulse facilitation. These parameters were not influenced by housing status. In contrast, long-term potentiation (LTP), which was no different between mutant (126 ± 4%, n=7) and WT (130 ± 4%, n=8) slices under SH, was enhanced (p<0.008) by EE in slices from WT mice (153 ± 6%, n=9), but not MSK1 KD
mice (125 ± 4%, n=8). These data, and a parallel study of hippocampus-dependent spatial reference and working memory (More' et al.; this meeting), implicate MSK1 as transducer of positive environmental stimulation into long-lasting structural and functional neuronal adaptations that underpin the enhanced cognition associated with enrichment. 1 Correa, S.A., et al., J Neurosci, 2012. 32 13039-51.


Poster

536. Learning and Memory: Genes, Signaling, and Neurogenesis I

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 536.13/BB47

Topic: F.02. Animal Cognition and Behavior

Support: BBSRC Grant BB/L00139X/1

WPH Charitable Trust

Title: MSK1 is a major contributor to the cognition-enhancing effects of environmental enrichment

Authors: *L. MORE1, L. PRIVITERA1, J. S. ARTHUR2, B. G. FRENGUELLI1;
1Life Sci., Univ. of Warwick, Coventry, United Kingdom; 2Col. of Life Sci., Univ. of Dundee, Dundee, United Kingdom

Abstract: Mice reared in an enriched environment (EE) show enhanced learning and memory, greater resilience to stressful situations, higher resistance to the addictive effects of drugs of abuse and improved recovery in both acquired and neurodegenerative brain injury. As such, considerable interest has arisen in the molecular mechanisms by which EE affects neuronal structure, function and cognition. We have previously shown that mice lacking the kinase activity of mitogen and stress activated kinase 1 (MSK1) did not display the enhancement of hippocampal synaptic transmission observed in wild-type (WT) mice after EE and showed a blunted increase in spine density [1]. We concluded that MSK1 may transduce some of the positive effects of EE into lasting structural and functional neuronal changes. This suggestion is made plausible given that 1) MSK1 is downstream of BDNF-activated TrkB receptors and 2) MSK1 has the ability to regulate gene expression via the phosphorylation of both CREB at S133 and histone H3 at S10. A number of these processes have been implicated in mediating the positive effects of EE. The present work investigated the extent to which EE improved
hippocampus-dependent spatial reference and working memory and cognitive flexibility in an MSK1-dependent manner during an early stage of life, i.e. from birth to 2.5-4 months of age. Our data show that the kinase activity of MSK1 is required for the full expression of the cognition-enhancing effects of EE: MSK1 kinase-dead mice showed less improvement in hippocampus-dependent spatial reference memory and reversal learning tasks in the Water-maze, and made fewer correct alternations during a spontaneous alternation task for spatial working memory. These data suggest that MSK1 is necessary for the full conversion of positive environmental stimulation into the enhancement of cognition. Parallel studies of synaptic transmission and plasticity (Privitera et al.; this meeting) will provide evidence for the cellular underpinnings of such enhancements of cognition.


Poster

536. Learning and Memory: Genes, Signaling, and Neurogenesis I

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 536.14/BB48

Topic: F.02. Animal Cognition and Behavior

Support: Whitehall Foundation

Title: A putative role for neurotensin receptor-2 in hippocampus

Authors: *M. A. THIBAULT1, L. MCQUADE2, H. WOODWORTH2, E. POTTER2, G. LEINNINGER2, A. ROBISON1;
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Abstract: The neuropeptide neurotensin regulates cells that express Neurotensin receptor-1 (NtsR1) or -2 (NtsR2), and this signaling system has been implicated in control of feeding, locomotor activity and learning. The lack of facile methods to identify NtsR-expressing cells, however, has limited understanding of their function. To overcome this, our lab generated mice that express green fluorescent protein (GFP) in NtsR2 cells (NtsR2GFP mice), thereby permitting identification of NtsR2 cells via immunofluorescent microscopy. Analysis of NtsR2GFP mice revealed many NtsR2-expressing neurons in the basal cell layer of the dentate gyrus. Since this part of the hippocampus gives rise to newly born glia and neurons during adulthood, we hypothesized that NtsR2 cells might arise from adult neurogenesis. Indeed, immunofluorescent labeling for markers of the various stages of neurogenesis identified mature...
NtsR2-expressing neurons as well as putative immature or “newly born” NtsR2 neurons. To determine whether new NtsR2-expressing neurons can be generated in vivo, we injected NtsR2GFP mice with BrdU, which selectively incorporates into newly generated cells in the adult nervous system. We find that many NtsR2-expressing dentate gyrus neurons are BrdU-positive, suggesting a potential role for NtsR2 in neurogenesis and/or neuronal maturation. We are interested in changes in gene expression that may regulate this process, and because mutant mice lacking the FosB gene have a malformed dentate gyrus as well as reduced neurogenesis, we sought to determine whether FosB gene products are found in NtsR2-expressing dentate gyrus neurons. Indeed, double labeling within NtsR2 neurons in the dentate gyrus reveals high levels of FosB within the nuclei. To determine the role of FosB in NtsR2 cells in dentate gyrus, we are now crossing NtsR2Cre mice with floxed FosB mice. Taken together, these studies reveal a possible role for neurotensin in the hippocampal neurogenesis and pave the way for future studies examining the role of NtsR2 learning and cognitive and psychiatric diseases.


Poster

536. Learning and Memory: Genes, Signaling, and Neurogenesis I

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 536.15/BB49

Topic: F.02. Animal Cognition and Behavior

Support: ERC grant no. 322744

    Swedish Research Council (2011-4544-84355-68)
    Swedish Brain Power
    Karolinska Institutet

Title: Nogo ligands, receptors, co-receptors and modulators in the developing and adult mouse brain

Authors: *G. SMEDFORS¹, K. WELLFELT², T. HJORTEHAMMAR², E. NORDLING², A. JOSEPHSON², L. OLSON², T. KARLSSON²;
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Abstract: The Nogo-system, a potent plasticity-restraining system in the CNS, is now in a broad sense known to include at least 16 proteins serving as ligands, receptors, co-receptors and modulators. When activated, it typically inhibits neuronal outgrowth through deregulation of the cytoskeleton. While much is known, no analysis of the expression of the complete system has been made. Therefore, we used quantitative in situ hybridization to localize transcriptional activity of Nogo, OMgp, MAG, Blys, NgR1-3, Lingo-1, Amigo 3, Troy, p75, S1PR2, Olfactomedin 1, Lotus, Lgl1 and Adam 22, from E15 to P730 mice. Nogo-A mRNA was strongly expressed in the E15 and E17 CNS. At P7 Nogo-A mRNA levels were relatively low in cortex, stronger in hippocampal CA1-3 layers and very low in the dentate gyrus. OMgp mRNA was not observed prenatally, weak at P0 with increasing levels in cortex and hippocampus at later stages. MAG was not robustly observed until P7 with a marked increase in white matter structures and in the form of large punctae in cortex and other gray matter areas at 30 days. At E15 and 17 NgR1 and Lingo 1 has a widespread expression in the CNS, while NgR2 is limited to the cortical mantle and DRGs and NgR3 is barely detectable. At P0 we find NgR2 mRNA to be more abundant than NgR1 and NgR3 mRNA in the cortical mantle, while at P7 NgR1 mRNA has increased. At P14, NgR1 and 2 genes remain active and Lingo-1 mRNA has increased in cortex and hippocampus. Interestingly, NgR3 and Lingo-1 are only weakly expressed in the dentate gyrus, and NgR2 mRNA is not detected in hippocampal area CA2. Ngr1-3 and Lingo-1 mRNA levels are maintained in the brain of 2 year old mice. The spatial and temporal patterns of transcriptional activity of these and additional genes listed above may reflect time windows and areas characterized by increased versus decreased structural plasticity as the brain develops, matures and ages.


Poster

536. Learning and Memory: Genes, Signaling, and Neurogenesis I

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 536.16/BB50

Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant F30 DA034480-01A1

NIH Grant MH083807

NIH Grant DA0270847
Title: Parameters for abolishing conditioned place preference for cocaine from running and environmental enrichment in male C57BL/6J mice

Authors: *M. L. MUSTROPH, H. PINARDO, J. R. MERRITT, J. S. RHODES; Neurosci., Univ. of Illinois Urbana-Champaign, Urbana, IL

Abstract: Recent evidence suggests that four weeks of voluntary wheel running can abolish conditioned place preference (CPP) for cocaine in male C57BL/6J mice, but key parameters need to be worked out before the mechanism behind the observed behavioral effects of running can be understood. The primary objective of this study was to determine the duration and timing of exposure to running wheels necessary to reduce CPP, and the extent to which the running per se influences CPP as compared to environmental enrichment without running. A total of 239 males were conditioned for 4 days twice daily with cocaine (10 mg/kg) and were then split into 7 different intervention groups prior to 4 consecutive days of CPP testing. The short sedentary group (SS; n=20) were housed in normal cages for 1 week. The short running group (SR; n=20) were housed with running wheels for 1 week. The short running group followed by a sedentary period (SRS; n=20) were housed in running wheels for 1 week and then normal cages for 3 weeks. The sedentary group followed by a short running period (SSR; n=20) were housed in normal cages for 3 weeks then 1 week with running wheels. The long sedentary group (LS; n=66) were housed in normal cages for 4 weeks. The long running (LR; n=66) group were housed with running wheels for 4 weeks. The long environmental enrichment group (EE; n=27) were placed in cages with multiple novel toys that were rotated on a weekly basis for 4 weeks.

Levels of running were similar in all running animals. Both running and environmental enrichment reduced CPP relative to sedentary groups; however, running tended to produce a greater reduction. One week of wheel running was sufficient to reduce CPP, and it did not matter whether the running was preceded or followed by a 3 week sedentary period. Results suggest the abolition of cocaine CPP from running is robust and occurs with as low as 1 week of intervention, and both increased physical activity and enrichment likely contribute to the phenomenon.


Poster

536. Learning and Memory: Genes, Signaling, and Neurogenesis I

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 536.17/BB51
INTRODUCTION: Phosphatase and tensin homolog deleted on chromosome 10 (PTEN) has been studied recently for its modulatory function on neurogenesis and synaptic plasticity, as it is important for key cellular processes, such as proliferation, migration and survival. However, because PTEN complete knockout is lethal, it is necessary to make use of different models in order to facilitate the study of its functions. In this context, conditional knockout through the Cre-lox system driven by the promoter of the neuron specific enolase (NSE) gene constitute an interesting tool for the assessment of PTEN neuronal functions.

METHODS: The aim of this study was to conduct a validation of the model of neuronal Pten partial deletion (NSE-PTEN+/−) in our laboratory and to initiate an investigation of still unknown effects of this deletion on behavior and metabolism. Therefore, 3-4 months old male mice from the NSE-PTEN+/− lineage had metabolic (body and brain weight, food consumption, glucose level and tolerance) and behavioral (anxiety and memory) parameters analyzed. Student t-test followed by Bonferroni post-hoc test was used for statistical analysis, with significance considered for p ≤ 0.05. RESULTS: We found that NSE-PTEN+/− mice had an increased ratio of food intake per body weight, however there were no significant effect on basal glycaemia or on glucose tolerance. There was also no significant difference on anxiety-associated parameters assessed through the open field test. NSE-PTEN+/− mice did not differ at the Morris water maze learning curve; however they spent less time on target quadrant on the 4h probe. Similarly, NSE-PTEN+/− mice showed a decreased latency in the inhibitory avoidance test. Furthermore, it was confirmed that NSE-PTEN+/− mice were macrocephalic, presenting augmented weight of total brain and cortex. DISCUSSION: with these results we validated the NSE-PTEN+/− mouse model in our laboratory, corroborating the occurrence of macrocephaly and cognitive impairment. Moreover, although glycaemia and glucose tolerance were not affected by the deletion, taking into consideration the increased food intake - which was not associated with augmented activity in the open field test - we reasoned the possibility of a modulatory role of neuronal PTEN on metabolism.
Poster

536. Learning and Memory: Genes, Signaling, and Neurogenesis I

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#/Poster#: 536.18/BB52

Topic: C.06. Developmental Disorders

Support: Max Planck Florida Institute

Title: Fear generalization in NLGN3R451C model of autism is associated with aberrant feedback inhibition in the lateral amygdala

Authors: B. UNAL, C. T. UNAL, *M. BOLTON; Disorders of Neural Circuit Function, Max Planck Florida Inst., Jupiter, FL

Abstract: Autism is a complex neurodevelopmental disorder characterized by social deficits, language impairment and repetitive behavioral patterns resulting from as yet undefined brain circuit imbalances. Given that the proper functioning of the amygdala is imperative for learning contingencies among emotionally charged stimuli and generation of adaptive emotional and social responses, we investigated amygdala circuit function in the neuroligin3R451C mouse model of autism. We found that the principal neurons of the lateral amygdala (LA) were hyper-connected by feedback inhibitory networks. The predominant pathology was enhanced inhibition of principal neurons by low threshold spiking (LTS) interneurons. Yet, the mice exhibited generalized fear in cued fear conditioning paradigms. Enhancing the power of inhibition onto LA principal neurons in wild type mice by pharmacogenetic activation of somatostatin-expressing low threshold spiking neurons resulted in fear generalization. Together these results provide evidence for dysregulation of inhibitory networks in the amygdala in a model of autism and highlight the importance of feedback inhibition in determining specificity of fear learning.

Title: The adenosine A2A agonist, CGS21680 reduces behavioral inflexibility and repetitive grooming in the BTBR Mouse

Authors: *M. E. RAGOZZINO¹, D. A. AMODEO², L. CUEVAS², J. A. SWEENEY³;
¹Psychology, Univ. Illinois Chicago, Chicago, IL; ²Psychiatry, Univ. of Texas Southwestern, Dallas, TX

Abstract: Autism spectrum disorder (ASD) is defined by social-communication deficits along with restricted interests and repetitive behaviors (RRBs). Based on findings in deer mice, we hypothesize that RRBs that include stereotyped movements and behavioral inflexibility result, in part, from under activation of the indirect pathway within basal ganglia circuitry. Thus, a treatment that enhances the indirect pathway should attenuate behavioral flexibility deficits and stereotyped movements. The BTBR T+ Ipr3tf/J (BTBR) mouse exhibits impairments in probabilistic reversal learning and repetitive grooming. Treatment with the adenosine 2A agonist CGS21680 is believed to facilitate activation of the indirect corticostriatal pathway. The current study tested whether facilitation of the indirect pathway through stimulation of adenosine 2A receptors attenuates a probabilistic reversal learning deficit and elevated grooming behavior in BTBR and B6 mice. For reversal learning, mice were tested in a spatial discrimination task using an 80/20 probabilistic reinforcement procedure. Acquisition and reversal learning occurred across two consecutive test sessions. Mice learned to obtain reinforcement from the “correct” spatial location (reinforced on 80% of trials) compared with the “incorrect” spatial location (reinforced on 20% of trials). Prior to the reversal learning phase, mice received a systemic injection of 0.01 mg/kg of CGS21680 or vehicle. As observed previously, vehicle-treated BTBR mice required significantly more trials to criterion than B6 mice during probabilistic reversal learning. CGS21680 treatment significantly reduced trials to criterion for reversal learning in BTBR mice compared to that of vehicle-treated BTBR mice and to a level that was comparable to vehicle-treated B6 mice. In BTBR mice, CGS21680 treatment improved reversal learning by significantly reducing regressive errors compared to vehicle treatment. For grooming behavior, mice received an intraperitoneal injection of CGS21860 0.01 mg/kg or vehicle prior to testing. Mice were individually placed in a clear plastic cage for 20 min. The first 10 min served as a habituation period. The second 10 min of testing a trained observer recorded cumulative time spent grooming all body regions. CGS21680 treatment also significantly reduced repetitive grooming in BTBR mice compared to vehicle treatment. Thus, treatment with an adenosine 2A receptor agonist may reduce RRBs in ASD and serve as a novel treatment in ASD.
Title: Effects of methylphenidate on state-action switching in rhesus monkey prefrontal cortex

Authors: *A. RAJALA, L. POPULIN, R. JENISON; Univ. of Wisconsin, Madison, Madison, WI

Abstract: The prefrontal cortex (PFC) is thought to play a central role in the ability to adapt to changing circumstances, including reward value. Methylphenidate (MPH), a psychostimulant used for the treatment of Attention Deficit Hyperactivity Disorder, increases catecholamine levels with the PFC and the basal ganglia, and is thought to interfere with such ability at high doses. Accordingly, we studied the effects of MPH on the performance of a reward-based switching task and tested the hypothesis that its effects on task performance result from changing reward signals within the PFC. Rhesus monkeys were tested using a reward-based oculomotor switching task consisting of alternating blocks of pro-saccades and anti-saccades without an overt cue alerting the switch. The scleral search coil technique was used to measure the subjects’ eye movements. Recordings were taken from single units in the dIPFC; MPH or vehicle was administered orally after a unit was isolated and baseline activity was recorded for 5-6 switching blocks. Recording was resumed immediately following drug administration. A variant of Q-learning was used to model the learning of action values following a switch in reward contingency. The subject must detect a true change in reward contingency and update the value of a particular action, either prosaccade or antisaccade, given their perceived current reward state. State-action pair values are learned within the computational model by updating the value of each action according to a temporal-difference rule. Parameters were fit to observed action
choices using the probability of choice logistic function over all trials. The association of trial-by-trial choice probability, action-value and reward prediction error with dIPFC single-unit discharge rates was evaluated using a Poisson-GLM under different doses of MPH. The dosage level had significant effects on the observed action choices, neural activity and the estimated parameter values of the Q-learning model. The difference in probability of choice following a state change was amplified at an optimal dose level compared with the control and higher dosage levels, and there was a decreased tendency to explore when optimally dosed. Both the model and neural prediction errors were enhanced under the optimal dose resulting in a faster recovery following the state switch. Finally, at higher dosage levels the subject was choosing actions randomly, with a near total failure to detect a switch in reward contingency, which correlated with the lack of differentiation of the reward prediction error in both the Q-learning model and neural signals.

Disclosures: A. Rajala: None. L. Populin: None. R. Jenison: None.

Poster

537. Prefrontal and Striatal Systems: Molecular Mechanisms and Connectivity

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 537.02/BB55

Topic: F.02. Animal Cognition and Behavior

Title: Stress-induced anxiety and c-fos immunoreactivity in adulthood following chronic juvenile methylphenidate exposure

Authors: *M. MCWATERS, E. ANDERSON, L. MATUSZEWICH;
Northern Illinois Univ., Dekalb, IL

Abstract: Methylphenidate (MPH), or Ritalin, is the most commonly prescribed psychostimulant for the treatment of Attention-Deficit Hyperactivity Disorder (ADHD). While the short-term clinical benefits of MPH have been established, the long-term effects after juvenile exposure has yielded mixed results (Galizio et al., 2009; Dow-Edwards et al., 2008). Long-term alterations of prefrontal cortex (PFC) catecholaminergic circuits may impact stress responses later in life as the same circuits that are targeted by MPH are activated during a stress response. In humans and animals, stress has been shown to increase anxiety-like behaviors (Reis et al., 2011). Therefore the current project tested whether chronic exposure to MPH during preadolescence altered anxiety-like behaviors and c-Fos immunoreactivity (IR) in the infralimbic prefrontal cortex (PFC) and hypothalamic paraventricular nuclei (PVN) either during basal conditions or to an acute restraint stress exposure. Juvenile male rats from PD21-35 were fed 2
mg/kg MPH or water on a vanilla wafer cookie daily. At approximately PD120, the rats were either exposed to restraint stress or left undisturbed and then tested in the elevated plus maze (EPM), a measure of anxiety. Following stress and EPM, rats were perfused and brains were sectioned for c-Fos IR. Preliminary findings suggest that there are no significant differences in locomotion in the open field between control and MPH treated rats in adulthood and that there are no significant differences in anxiety-like behaviors as a result of drug condition or stress condition. However, there was a significant interaction between drug and stress conditions (p=.038). In rats exposed to restraint stress, juvenile MPH decreased anxiety-like behavior compared to rats that received water. Whereas in rats not exposed to restraint stress, MPH treated rats had greater anxiety-like behaviors compared to control rats. Drug condition did not significantly affect c-Fos IR in the infralimbic PFC or PVN. Restraint stress did not significantly affect c-Fos IR in PVN, but did significantly increase c-Fos IR in the infralimbic PFC (p=.013), suggesting that the effect of stress may be more pronounced in the PFC, rather than the PVN. Overall, the long-term effects of juvenile MPH treatment differed in the expression of anxiety-like behaviors depending upon the acute exposure to stress, but the pattern of c-Fos IR did not parallel the behavior. Understanding potential long-term effects of juvenile MPH exposure may be essential for the development of better treatment strategies for individuals with ADHD.

Disclosures: M. McWaters: None. E. Anderson: None. L. Matuszewich: None.

Poster
537. Prefrontal and Striatal Systems: Molecular Mechanisms and Connectivity

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 537.03/BB56

Topic: F.02. Animal Cognition and Behavior

Support: NIDA R01DA031695

Title: Cocaine self-administration enhances response-outcome encoding in dorsal striatum

Authors: *A. C. BURTON, G. B. BISSONETTE, A. C. ZHAO, P. K. PATEL, M. R. ROESCH;
Dept. of Psychology, Univ. of Maryland, Col. Park, College Park, MD

Abstract: Drug addiction is commonly associated with maladaptive decision-making and habit-like behaviors. The development of addiction is thought to reflect a transition from response-outcome (goal-directed) to stimulus-response (habit) driven behavior, functions which are thought to be under the control of ventral (VS) and dorsolateral striatum (DLS), respectively.
Previously, we have shown that VS dysfunction due to lesion, irrespective of drug use, enhanced stimulus-response encoding in DLS while rats performed an odor-guided choice task. Here, we ask if previous cocaine self-administration in rats alters neural encoding in DLS in the same task. To the best of our knowledge, no study has conclusively shown enhanced stimulus-response encoding in DLS after chronic cocaine use. To address this issue, we implemented a two-week cocaine (cocaine group) or sucrose pellet (control) self-administration protocol with a one-month withdrawal period and then recorded from single neurons in DLS while rats performed an odor-guided choice task where reward value was manipulated by changing the delay to or size of reward. We describe data showing that after going through withdrawal, the cocaine group was more impulsive during performance of the task especially during delay blocks. Surprisingly, we show an increase in the number of neurons encoding response-outcome associations in DLS rather than stimulus-response associations. These results suggest that cocaine self-administration impacts behavior and encoding in DLS. Specifically, it suggests that impulsive behavior observed after chronic-cocaine use does not reflect an enhancement of stimulus-response encoding, but instead, an over representation of response-outcome encoding in DLS.


Poster

537. Prefrontal and Striatal Systems: Molecular Mechanisms and Connectivity

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 537.04/BB57

Topic: F.02. Animal Cognition and Behavior

Support: Fondecyt 1130042

Nucleo Milenio P10063-F

Title: H3 receptor inactivation normalized disrupted histamine transmission during sexual motivation

Authors: *M. E. RIVEROS1,2, F. TORREALBA1;
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Abstract: Chronically stressed animals have depressive like symptoms such as reduction in defensive behaviors and reduction in sexual or food motivation. We have proposed that the
histaminergic system has a role in motivated behavior implementation directed by its principal cortical input, the infralimbic cortex (IL), and shown that there is histamine release in the medial prefrontal cortex during appetitive behavior that is proportional to the number of lever presses for food (motivation). Chronically stressed animals have reduced histamine release during appetitive behavior for food together with reduced motivation, but an intra-peritoneal injection of the H3 antagonist thioperamide increases motivation for palatable food in stressed rats. Stress is known to induce histamine release, and it is possible that chronic histamine overstimulation by chronic stress alter histamine transmission. Here we wanted to explore if chronic stress altered histamine response to acute stress and to study the effect of chronic immobilization stress on sexual motivation in relation to histamine release in the prefrontal cortex and nucleus accumbens (nAcc) of male rats. Tail suspension or receptive-female urine sniffing test (FUST) induced histamine release in the prefrontal cortex of control animals but not stressed animals. FUST reduced histamine release in the nAcc of control animals but not stressed animals. Intraperitoneal thioperamide in stressed animals before FUST increased histamine release in the IL and reduced it in the nAcc. In conclusion, chronic stress reduced histamine release in IL in response to acute stress or appetitive sexual stimulus. The systemic inactivation of H3 receptor normalized the response of stressed rats during sexual motivation in FUST.

Disclosures: M.E. Riveros: None. F. Torrealba: None.
including ventral striatum (vStr), in aggressive behavior mice. DeltaFosB is a truncated splice variant of FosB protein that plays a key role in the function of the brain reward system is increased in the vSTR of aggressive mice. Viral overexpression of deltaFosB in the vStr of aggressive mice intensifies aggression, whereas suppression of FosB expression diminishes aggressive behavior. These findings support the notion that deltaFosB in vStr promotes aggressive behavior. Considering the role of deltaFosB in other reward-related behaviors, such as drug addiction, sexual pleasure and alcohol drinking, our data may point to a new role of deltaFosB in regulating motivational aspects aggressive behavior in mice.


**Poster**

537. Prefrontal and Striatal Systems: Molecular Mechanisms and Connectivity

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program/#Poster#:** 537.06/BB59

**Topic:** F.02. Animal Cognition and Behavior

**Support:** NIMH grant MH096890

**Title:** Setdb1 histone H3K9 methyltransferase knockout elicits anxiety phenotype

**Authors:** *B. JAVIDFAR, Y. JIANG, S. AKBARIAN;* Icahn Sch. of Med. At Mount Sinai, New York, NY

**Abstract:** The histone H3-lysine 9 residue is a major target of the methyltransferase Setdb1 (Set domain, bifurcated 1), also known as Kmt1e, in the adult brain. Setdb1 trimethylation of H3-lysine 9 is associated with repressive chromatin remodeling and downregulation of a select group of target genes, but the behavioral effects of Setdb1 downregulation remain unknown. We previously reported an antidepressant-like phenotype in adult transgenic mice with increased Setdb1 expression in the forebrain. Here, we report that conditional knockout of Setdb1 via AAV-Cre injections in prefrontal cortex results in an anxiety-like phenotype. In conjunction with our previously reported data, these findings suggest that Setdb1 has a profound effect on the modulation of anxiety-related behavior.

**Disclosures:** B. Javidfar: None. Y. Jiang: None. S. Akbarian: None.
**Poster**

**537. Prefrontal and Striatal Systems: Molecular Mechanisms and Connectivity**

**Location:** Hall A  
**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 537.07/BB60

**Topic:** F.02. Animal Cognition and Behavior

**Support:** 5R37-MH-073853  
5U-19-MH-082441

Pall Family foundation

Sidney R. Baer Jr. Foundation

**Title:** A unique dual cortico-striatal action of a beta-arrestin biased dopamine D2 receptor ligand

**Authors:** *N. URS*, S. M. GEE, T. F. PACK, J. D. MCCORVY, T. EVRON, B. L. ROTH, P. O’DONNELL, M. G. CARON  

**Abstract:** The dopamine hypothesis of schizophrenia postulates hypodopaminergia in the prefrontal cortex and hyperdopaminergia in the striatum. Current antipsychotics effectively reverse excess striatal activity, but do not fully reverse cortical deficits, a problem we address here. Using neuron-specific β-arrestin2 (βarr2)-knockout mice and the β-arrestin-biased D2 receptor (D2R) ligand UNC9994A (94A), we show that βarr2 antagonism in striatal D2R+ neurons is sufficient for antipsychotic activity against amphetamine. However, for antipsychotic activity against phencyclidine, 94A displayed a unique regional selectivity, which can be attributed to enhanced cortical D2R/βarr2 agonism through increased excitability of cortical fast-spiking interneurons (FSI). The switch from antagonism to agonism is consistent with higher cortical expression of βarr2 and GPCR Kinase2 (GRK2) compared to striatum. Therefore, unlike current antipsychotics, β-arrestin-biased D2R ligands that behave as agonists in the cortex but antagonists in the striatum may be sufficient for clinical antipsychotic efficacy, with a superior ability to correct cortical hypodopaminergia. Such a drug could have profound implications in the treatment of cognitive and negative symptoms of schizophrenia in addition to ameliorating psychosis.
**Disclosures:**  
N. Urs: None.  
S.M. Gee: None.  
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J.D. McCorvy: None.  
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M.G. Caron: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Acadia Stock.  
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**Poster**

**537. Prefrontal and Striatal Systems: Molecular Mechanisms and Connectivity**

**Location:** Hall A  
**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM  
**Program/#Poster#: 537.08/BB61  
**Topic:** F.02. Animal Cognition and Behavior  
**Title:** Phosphodiesterase 2A inhibition and impulsivity  
**Authors:**  
P. R. A. HECKMAN1,2, A. BLOKLAND3, J. RAMAEKERS2, J. PRICKAERTS1;  
1Dept. of Psychiatry & Neuropsychology, 2Dept. of Neuropsychology & Psychopharmacology, Maastricht Univ., Maastricht, Netherlands  

**Abstract:** Impulsivity is a multifaceted concept that comes in many different forms. Impulsive behavior is generally divided into ‘impulsive actions’, i.e. an inability to inhibit a response, ‘impulsive choices’, i.e. a distorted judgment with respect to choosing between two different outcomes, and ‘reflection impulsivity’, the ability to evaluate available information prior to deciding. ‘Impulsive actions’ are generally subdivided into ‘action restraint’, i.e. inhibiting a prepotent, inappropriate response and ‘action cancellation’, i.e. response inhibition or volitional control over responding once the response has been initiated. ‘Impulsive choices’ may be subdivided into delay-, uncertainty-, and effort-based decision making. All concepts of impulsivity seem to find their origin in frontostriatal circuitry. Dopamine (DA) is an important moderator of this circuitry. The extracellular effect of DA is largely mediated through the cAMP/PKA signaling cascade. Importantly, the cGMP/PKG signaling cascade is known to modulate the cAMP/PKA cascade in both the striatal direct D1 and indirect D2 pathway neurons. These cascades are thus a potential target for pharmacological intervention of DAergic signaling in impulse control disorders, like addiction or ADHD. Phosphodiesterase type 2 (PDE2A), which degrades both the intracellular messengers cAMP and cGMP, is known to be present in the frontal cortex and the striatum. In the current project we therefore investigated whether the PDE2A inhibitor BAY 60-7550 could reverse both a systemic amphetamine-induced deficit in...
impulse control, as well as impulsivity caused by induction of 6-OHDA lesions in medial prefrontal cortex (mPFC), in operant chambers in adult rats by means of conditioned reaction time task schedules of reward. We hypothesized that based on the major presence of PDE2 in the frontal cortex and either the direct or indirect pathway, the effect of the PDE2 inhibitor increases or decrease impulsivity, respectively.

Disclosures: P.R.A. Heckman: None. A. Blokland: None. J. Ramaekers: None. J. Prickaerts: None.

Poster

537. Prefrontal and Striatal Systems: Molecular Mechanisms and Connectivity

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 537.09/BB62

Topic: F.02. Animal Cognition and Behavior

Title: Cholinergic modulation of nicotine-evoked glutamate release within the dorsal striatum

Authors: *D. YOUNG, R. KOZAK, W. HOWE; Pfizer Inc, Cambridge, MA

Abstract: Aberrant function of the neural mechanisms controlling motivation and goal-directed action contributes to the manifestation of the cognitive and negative symptoms of schizophrenia, as well as the range of impulsive and apathetic behaviors in neurodegenerative disorders such as Parkinson’s and Alzheimer’s (Barch and Dowd, 2010, Der-Avakian and Markou, 2012). While much work has focused upon dopaminergic signaling within the ventral striatum in the control of such behavior, collective evidence suggests that activity in the dorsal striatum also plays a causal role. The topographic organization of glutamatergic input to DS is central to its function and is hypothesized to be modulated locally by cholinergic interneurons (e.g. Lovinger, 2010). The present experiments make use of enzyme-selective biosensors for sub-second monitoring of neurotransmitter release (~300 ms temporal resolution) to determine the unique contributions of the heteromeric α4β2 and homomeric α7 nAChRs to cholinergic modulation of glutamate release in the dorsal striatum. Blockade of α7 receptors by MLA resulted in a significant attenuation of nicotine-evoked glutamate release (n=5, 50% reduction in evoked glutamate release), while blockade of α4β2 receptors resulted in a robust increase (n=5, 300% above baseline). As α4β2 sit presynaptically on dopaminergic inputs to the dorsal striatum, on-going follow up studies seek to test the hypothesis that nicotine-evoked DA release acts via presynaptic D2 receptors to further modulate glutamate release. The combined results of these studies seek to add to our understanding of the micro-circuitries that mediate excitatory input to the dorsal striatum.
Unbalanced cholinergic-dopaminergic gating of glutamatergic input to the dorsal striatum may contribute to deficits in motivation and other cognitive processes that are attributed to myriad neuropsychiatric disorders.

**Disclosures:** D. Young: A. Employment/Salary (full or part-time); Pfizer Inc. R. Kozak: A. Employment/Salary (full or part-time); Pfizer Inc. W. Howe: A. Employment/Salary (full or part-time); Pfizer Inc.

**Poster**

537. Prefrontal and Striatal Systems: Molecular Mechanisms and Connectivity

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 537.10/BB63

**Topic:** F.02. Animal Cognition and Behavior

**Support:** Medical Research Council

Royal Society

Wellcome Trust

**Title:** Catechol-O-methyltransferase affects striatal dopamine transmission and modulates the influence of cue salience on associative learning

**Authors:** *A. HUBER*¹², L. OIKONOMIDIS¹, J. GAUNT¹, E. M. TUNBRIDGE², M. E. WALTON¹;

¹Dept. of Exptl. Psychology, ²Dept. of Psychiatry, Univ. of Oxford, Oxford, United Kingdom

**Abstract:** The enzyme catechol-O-methyltransferase (COMT) plays an important role in the breakdown of dopamine in prefrontal cortex, but has been presumed to have little effect over striatal dopamine. However, several neuroimaging studies have showed an influence of variation in COMT genotype on striatal BOLD signals, particularly during reward-guided learning and decision making. To investigate further the effect of COMT genes on striatal dopamine-dependent behaviours and striatal dopamine release, we used combinations of behavioural testing and fast-scan cyclic voltammetry in a transgenic mouse model of the Val158Met polymorphism found in the human COMT gene. In one set of experiments, mice were implanted with carbon fibre electrodes in the nucleus accumbens core to determine the influence of COMT genotype on stimulus-evoked dopamine release. Mice were initially trained on a variable interval schedule to gain a standard reward size. Subsequently, dopamine release was recorded in a session where standard reward trials were intermixed with decreased or increased reward size trials. While
reward delivery elicited rapid increases in dopamine that scaled with reward size in both groups, there was a significant attenuation in this scaling in the Met-COMT mice, in particular for smaller-than-expected rewards (i.e., negative prediction errors). In a second set of experiments, we tested whether variations in COMT genotype affects appetitive learning, which is known to depend on intact striatal dopamine. Groups of Met-COMT mice and wild type littermates (WT) were first trained on a Pavlovian conditioning paradigm where auditory cues - a 3kHz tone or white noise - were followed by either the delivery of reward or no reward. While both groups on average learned at an equal rate to associate the CS+ with reward, closer analysis showed that the speed of acquisition was significantly faster in Met-COMT mice when the white noise was the CS+, but significantly slower when instead the tone was the CS+. Further experiments demonstrated that learning rates were more strongly modulated by assignment of CS+ cue salience in the Met-COMT mice. Together, these results suggest subtle but influential roles for COMT genotype on reward-elicited dopamine release and reward-guided learning.


Poster

537. Prefrontal and Striatal Systems: Molecular Mechanisms and Connectivity

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 537.11/BB64

Topic: F.02. Animal Cognition and Behavior

Support: Wellcome Trust
Royal Society
Clarendon Fund

Title: Contributions of COMT and DAT to regulation of phasic dopamine release and reward-guided behavior

Authors: *C. KORN, C. VAGNONI, M. WALTON, E. TUNBRIDGE;
Oxford Univ., Oxford, United Kingdom

Abstract: Fine temporal regulation of dopamine transmission is critical to dopaminergic mediation of behavior. Dopamine can be cleared from the synapse by several mechanisms. Enzymatic degradation involving catechol-O-methyltransferase (COMT) is one of the main means of clearing cortical dopamine, which modulates executive functions. Recycling by the
dopamine transporter (DAT) predominates in the striatum, where dopamine regulates reward-guided behavior. However, evidence from human functional imaging studies demonstrates interactive effects of COMT and DAT genotype, suggesting that this segregation is not so clear-cut. Given the interdependence of mesolimbic and mesocortical circuitry and the presence of COMT (albeit at relatively low levels) in striatum, it is possible that COMT may interact with DAT to influence dopamine transmission and dopamine-dependent behaviors. Therefore, we used in vivo electrochemical recording, pharmacology, and behavioral testing to investigate the contributions of COMT and DAT to regulation of dopamine transmission and reward-guided behavior in male mice. Fast-scan cyclic voltammetry was used to monitor the effects of a DAT blocker (GBR-12909, 6mg/kg) and a COMT inhibitor (tolcapone, 30mg/kg) on dopamine transmission evoked by electrical stimulation of the ventral tegmental area, in vivo, in anaesthetized mice. Systemic blockade of DAT significantly increased the peak height of evoked dopamine transients in the nucleus accumbens (NAcc) (n = 14) but not in the medial frontal cortex (MFC) (n = 8). By contrast, COMT inhibition did not significantly alter the peak height of evoked dopamine in either NAcc (n = 14) or MFC (n = 9). However, preliminary analyses of kinetic parameters suggest that there may be interactive effects of COMT inhibition and DAT blockade on dopamine transmission in these regions not captured by the assessment of peak height. Concurrent behavioral tests in awake animals found that only systemic administration of the DAT blocker, but not of the COMT inhibitor, increased motivation to work for reward in a progressive ratio paradigm (n = 22). Thus far, the results of this study support prevailing ideas about how DAT blockade alters striatal dopamine transmission and associated behaviors and do not yet offer clear evidence of COMT-DAT interactions in the regulation of phasic dopamine release or reward-guided behavior.

**Disclosures:** C. Korn: None. C. Vagnoni: None. M. Walton: None. E. Tunbridge: None.
**Abstract:** mTORC1 pathway mediates dendritic protein synthesis, synaptic plasticity and thus contributes to learning and memory [1]. Alcohol addiction is a maladaptive form of learning and memory processes [2]. We previously found that excessive alcohol drinking activates mTORC1 in the rodent nucleus accumbens (NAc), a key component of the brain reward system [3]. We further reported that inhibition of mTORC1 by systemic or intra-NAc administration of its specific inhibitor, rapamycin, attenuated alcohol-related behaviors in rodents, including the expression of alcohol-induced locomotor sensitization and place preference, home-cage excessive drinking, as well as operant self-administration and seeking [4]. Finally, we found that systemic administration of rapamycin also disrupted the reconsolidation of alcohol-associated memories, leading to suppression of alcohol relapse in rats [5]. Here we report that mTORC1 is activated in the orbitofrontal (OFC) but not in the prefrontal cortex of rats that consumed 20% alcohol for 8 weeks in an intermittent choice paradigm. The OFC is a critical component of the corticostriatal circuitry that dynamically coordinates the balance between goal-directed and habitual actions, and impairment in this balance as well as OFC dysfunction are associated with alcohol addiction. Thus, we hypothesized that mTORC1 in the OFC is required for the transition from goal-directed to habitual responding for alcohol. To test this possibility, we used an operant paradigm in which rats with a history of excessive alcohol drinking self-administered 20% alcohol under a random ratio (RR) or a random interval (RI) schedule of reinforcement. Then, we used a revaluation test and showed that alcohol devaluation reduces responding for alcohol in RR-trained but not RI-trained rats, revealing goal-directed and habit-driven behavior, respectively. Next, we tested whether mTORC1 inhibition in the OFC alters the sensitivity to devaluation in RR- and RI-trained rats. We found that intra-OFC infusion of rapamycin 3 hrs prior the devaluation test did not affect the goal-directed alcohol responding in RR-trained rats but restored the sensitivity to devaluation in RI-trained rats. Together, these data suggest that alcohol-mediated activation of mTORC1 in the OFC contributes to the expression of habitual alcohol responding.


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**Poster**

537. Prefrontal and Striatal Systems: Molecular Mechanisms and Connectivity

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM
**Program#/Poster#:** 537.13/BB66

**Topic:** F.02. Animal Cognition and Behavior

**Support:** CCTSI Pilot Grant CNSBI-14-65

**Title:** GCN5 enzymatic activity is required for normal corticostriatal development and function

**Authors:** *J. WILDE, L. NISWANDER;*  
Pediatrics, UC Denver, Aurora, CO

**Abstract:** Defects in corticostriatal circuits and the pathways necessary for their development and maintenance are thought to underlie numerous psychiatric and neurological diseases. GCN5 is an acetyltransferase with established functions in nervous system development and memory, but it is unknown whether it plays a role in corticostriatal development and function or behavior. We find that mice heterozygous for an enzymatically inactive form of GCN5 (Gcn5hat+/−) exhibit phenotypes similar to those seen in Slitrk5−/− mice, an established model for OCD. Specifically, Gcn5hat+/− mice develop severe self-injurious behavior and show signs of anxiety in multiple behavioral assays. In order to further investigate these phenotypes, we developed a longitudinal study examining behavior, molecular markers, cellular morphology, and volumetric changes in Gcn5hat+/− mice over the course of 15 months. Molecular clues generated by microarray analysis of forebrain tissue from E10.5 embryos completely lacking GCN5 activity revealed disruption of multiple processes implicated in OCD and mood disorders, indicating that developmental mechanisms may underlie the phenotypes seen in heterozygous adults. By 6 months of age, Gcn5hat+/− mice show significant behavioral differences in a marble-burying assay, with no apparent anxiety phenotypes. Mutants display significantly greater variability in behavioral assays and progress to show signs of anxiety by 8 months of age. Furthermore, 12-month-old Gcn5hat+/− mice show significantly decreased complexity of striatal medium spiny neurons, increased neuronal activity in the orbitofrontal cortex, and evidence of decreased striatal volume, mirroring the phenotypes seen in Slitrk5 mutants. Together, our data suggest that GCN5 acetyltransferase activity is required for proper development and function of corticostriatal circuits and that alteration of GCN5 activity may underlie some behavioral disorders.

**Disclosures:** J. Wilde: None. L. Niswander: None.

**Poster**

**537. Prefrontal and Striatal Systems: Molecular Mechanisms and Connectivity**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 537.14/BB67
Title: Corticotropin-releasing factor (CRF) impairs prefrontal cortex-dependent cognitive processes

Authors: *S. HUPALO*¹,², R. C. SPENCER², C. W. BERRIDGE²;¹Neurosci. Training Program, ²Psychology, Univ. of Wisconsin - Madison, Madison, WI

Abstract: The prefrontal cortex (PFC) regulates cognitive processes critical for flexible, goal-directed behavior. Dysfunction of PFC-dependent cognition is associated with a variety of psychopathologies. It has long been known that corticotropin-releasing factor (CRF) and CRF receptors are prominent in the PFC. However, despite decades of research into the neurobiology of CRF, the cognitive actions of CRF signaling in the PFC remain largely unknown. To address this, we tested the hypothesis that CRF modulates PFC-dependent cognition. We first examined the effects of intracerebroventricular (ICV; 0.1, 0.2, 1, 3 µg) and intra-PFC (25, 50, 100, 250 ng/hemisphere) CRF infusions on performance in a task of spatial working memory in rats. ICV and intra-PFC infusions of CRF elicited a robust and dose-dependent impairment in task performance. Interestingly, the cognitive effects of CRF signaling in the PFC were topographically organized, such that CRF infusions into the caudal, but not rostral, dorsomedial PFC (dmPFC) impaired working memory performance. Subsequent studies investigated whether endogenous CRF signaling modulates working memory using the CRF antagonist, D-Phe-CRF. Both ICV (2, 4, 10 µg) and intra-caudal dmPFC (50, 200 ng/hemisphere) infusions of this antagonist dose-dependently improved working memory performance. Given all FDA approved treatments for ADHD improve working memory performance in rodents, monkeys and humans, these observations raise the hypothesis that CRF antagonists may be effective in treating ADHD and other disorders associated with PFC-dependent cognitive dysfunction. Additional studies examined whether CRF modulates PFC-dependent cognition more broadly. Specifically, we tested the effects of ICV and intra-PFC CRF infusions in a signal detection task of sustained attention using doses similar to those in the working memory studies. ICV CRF dose-dependently impaired performance in this task. However, in contrast to that seen with working memory, CRF infusions into both rostral and caudal dmPFC subfields had no effect in sustained attention. This indicates that the impairing effects of ICV CRF in sustained attention are not due to CRF action in the PFC. Thus, CRF signaling in the PFC differentially modulates distinct PFC-dependent processes. Ongoing studies are examining the degree to which ICV administration of a CRF antagonist improves sustained attention similarly to working memory. Collectively, this research provides novel insight into the neurobiology of PFC-dependent cognition and may have relevance for treating psychopathologies associated with PFC dysfunction.
Disclosures:  S. Hupalo: None.  R.C. Spencer: None.  C.W. Berridge: None.

Poster

537. Prefrontal and Striatal Systems: Molecular Mechanisms and Connectivity

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 537.15/BB68

Topic: F.02. Animal Cognition and Behavior

Support: T32 NIAAA AA07462

Title: Genetic and pharmacologically mediated changes in neural synchrony across the mesocorticolimbic dopamine system during alcohol consumption

Authors: *A. M. MCCANE¹, S. AHN², L. RUBCHINSKY¹, S. S. JANETSIAN¹, D. N. LINSENBARDT¹, C. L. CZACHOWSKI¹, C. C. LAPISH¹;
¹Psychology, Indiana University-Purdue Univ. Indianapolis, Indianapolis, IN; ²Sch. of Math and Stat Sci., Arizona State Univ., Tempe, AZ; ³Dept. of Mathematical sciences, Indiana University-Purdue University Indianapolis, Indianapolis, IN

Abstract: Alterations in the mesocorticolimbic (MCL) dopamine (DA) pathway have been implicated in alcohol use disorder (AUD). The neural responses to stimuli that predict drug availability have been postulated as a clinically relevant target for AUD therapies. The present experiments sought to first characterize changes in network-level neural activity of the MCL system during cued drug seeking in a rodent model of AUD. Secondly, we sought to determine the consequences of modulating cortical DA on neural synchrony and associated changes with alcohol seeking behaviors.

Subjects were alcohol preferring (P) and Wistar rats engaged in a Pavlovian conditioning paradigm where the illumination of a stimulus light signaled the availability of an ethanol solution. Upon reaching stable responding (i.e, approach behavior) in this task, animals were implanted with microelectrodes in VTA, PFC, and NA to acquire local field potentials (LFPs) during the conditioning task. LFPs were digitally filtered in the theta band (5-11 Hz). Recordings were segregated into three 10-second epochs (before CS, during US, after US) and the phase locking index $\gamma$ was calculated as a measure of the strength of phase locking between signals from any two brain regions. The enzyme catechol-O-methyltransferase (COMT) is the primary mechanism of DA metabolism in the PFC. To assess how cortical DA modulation impacted neural synchrony and corresponding behavioral measures, the COMT inhibitor Tolcapone was administered prior to conditioning sessions on drug test days. In both strains, overall synchrony was significantly stronger between the NA and PFC than the PFC-VTA or VTA-NA. However, P rats showed reduced NA-PFC synchrony relative to Wistars. During
drinking epochs, robust increases in theta synchrony were observed in P rats, and to a lesser extent in Wistars. A transient decrease in VTA-NA synchrony during drinking was also observed in P rats but not Wistars. Administration of Tolcapone reduced both alcohol consumption and PFC-NA synchrony in P rats but not Wistars. Alterations in PFC-striatal connectivity has been reported in individuals with AUD. Impaired frontal-striatal connectivity is correlated with both alcohol craving and dependence severity. Moreover, alterations that occur between the PFC and NA are hypothesized to be linked to an enhanced reactivity to drug paired stimuli in addicts. These data implicate aberrant connectivity between the PFC and NA and suggest the clinical utility of COMT inhibition as a therapy for AUD.


Poster

537. Prefrontal and Striatal Systems: Molecular Mechanisms and Connectivity

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 537.16/BB69

Topic: F.02. Animal Cognition and Behavior

Title: Which anatomical substrate is reflected by MRI-based connectomics? lessons from the Allen mouse brain connectome atlas

Authors: *V. ZERBI*¹, J. GRANDJEAN², Z. PRÖHLE³, M. RUDIN²,⁴, N. WENDEROTH¹;
¹Neural Control of Movement Lab, ETH Zurich, Zurich, Switzerland; ²Inst. for Biomed. Engineering, ETH and Univ. Zurich, Zurich, Switzerland; ³Dept. of Physics, ETH Zurich, Zurich, Switzerland; ⁴Inst. of Pharmacol. and Toxicology, Univ. Zurich, Zurich, Switzerland

Abstract: Resting-state fMRI (rs-fMRI) and diffusion weighted imaging (DWI) are widely used MRI techniques in the field of neuroscience. These MRI tools offer insights into the brain’s functional and structural connectivity by measuring coherence of intrinsic neural activity and axonal anatomical pathways, respectively. However, for both modalities connectivity measurements are indirect and it is not clear in how far they reflect the anatomical connectome. For example, regions that are functionally connected based on rs-fMRI do not always have direct structural connections as measured with DWI-based tractography, as found for the extra-striate visual cortices(1). While an intermediate region could form the anatomical relay between these areas, such a putative region may not be apparent from the functional maps of these networks. These observations raise the question which anatomical substrate is reflected by the functional and structural networks commonly observed via MR based methods in humans and in rodents.
Here we investigate the relationship between MRI-based connectomics analyses and monosynaptic connectivity measures in the mouse brain. The connectivity data from the Allen Brain Institute derived from viral injection experiments(2) provides unique information on monosynaptic connectivity in the mouse brain. Our goal in this study is to directly compare the results from connectome analyses obtained either by rs-fMRI and DWI-based tractography, with the anatomical ground truth provided by the Allen Brain Institute. Rs-fMRI and DWI datasets were acquired in C57BL/6J mice on a 9.4T scanner (Bruker BioSpec 94/30) equipped with a gradient system capable of a maximum gradient strength of 400 mT/m with an 80 ms rise time. Best-practice methods are applied to obtain resting-state whole brain connectivity maps(3), using over 400 injection areas from the Allen institute as seed regions. A structural connectome is also obtained from gray matter ROIs in the mouse brain, using anatomical constrained probabilistic tractography and SIFT correction for white matter density(4). The resulting connectome matrices will be directly compared to the viral injection-based connectivity maps from the Allen institute. The results of this study will link MRI measurements of structural and functional connectivity to the anatomical ground truth, which is an important step to better interpret MRI-based studies of the connectome in rodents and in humans. 1) van den Heuvel et al. Hum Brain Mapp. 2009 Oct;30(10):3127-41 2) Oh et al. Nature. 2014 Apr 10;508(7495):207-14 3) Grandjean et al. Neuroimage. 2014 Aug 28;102P2:838-847. 4) Smith et al. Neuroimage. 2013 Feb 15;67:298-312.

Disclosures: V. Zerbi: None. J. Grandjean: None. Z. Pröhle: None. M. Rudin: None. N. Wenderoth: None.

Poster

537. Prefrontal and Striatal Systems: Molecular Mechanisms and Connectivity

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Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 537.17/BB70

Topic: F.02. Animal Cognition and Behavior

Support: MRC Grant MC-A060-5PQ14

Title: Functional and structural changes associated with recovery of function following lesions to principal sulcus in macaques

Authors: *M. AINSWORTH1,3, H. BROWNCROSS2, D. J. MITCHELL3, A. S. MITCHELL2, J. SALLE2, M. J. BUCKLEY2, J. DUNCAN2,3, A. H. BELL2,3, 2Dept. of Exptl. Psychology, 1Oxford Univ., Oxford, United Kingdom; 3MRC Cognition and Brain Sci. Unit, Cambridge, United Kingdom
Abstract: It has been shown that there can be recovery of function following damage to certain areas of cortex, the nature and degree of recovery depending on the specific area. However, it is currently unknown how brain areas outside the damaged region(s) compensate to drive this recovery. Here we collected BOLD fMRI and structural imaging data across multiple time points following lesions to the left and right principal sulcus in two macaque monkeys. The objective was to examine the functional and structural changes that occur over an extended period in brain areas following sequential lesions to the prefrontal cortex. Monkeys were trained to perform two delayed-match tasks. In the “match-to-object” task, a target stimulus was presented, which the monkey was required to touch. After a variable delay (2-16s), the same stimulus appeared in a random location embedded within a number of distracting stimuli, and the monkey was required to again touch the target stimulus, irrespective of its location. In the “match-to-location” task, the monkey was required to indicate the location of the previously presented target stimulus, irrespective of the identity of the stimulus currently presented there. Once animals had reached a criterion level of performance on both tasks (>70%), we collected functional and structural imaging data under general anaesthesia at periodic intervals (4 times approximately once every 3 weeks). Several days prior to each scanning session, the animals were tested on both the object and location tasks (2-3 testing sessions for each task across 2-3 days). After four such testing sessions, the animals received a lesion to both banks of the left principal sulcus. Following a post-operative recovery period, we resumed periodic testing and scanning sessions (4 times approximately once/3 weeks). Once the animals had returned to pre-lesion performance levels, they received a lesion to the right principal sulcus. The animals were once again tested and scanned (4 times, approximately once every 3 weeks). Animals showed impairment followed by eventual recovery in both tasks following each lesion. The drop in behavioural performance was accompanied by disturbed functional connections (as measured by resting state correlations) between specific regions in both hemispheres that were identified to be strongly functionally connected to the principal sulcus in a control dataset. Following the lesions, we also observed reduction in grey matter volume across multiple brain areas. These data suggest that, following a focal frontal lobe lesion, behavioural impairments reflect not just the functions of the lesioned area, but disturbance of an extensive cortical network.


Poster

537. Prefrontal and Striatal Systems: Molecular Mechanisms and Connectivity

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 537.18/BB71
Title: Characterizing circuits for positive and negative social interactions in mPFC

Authors: *L. CHUNG, K. SAKURAI, S. ZHAO, F. WANG;
Neurobio., Duke Univ., Durham, NC

Abstract: Identifying specific neurons relevant to complex behaviors is the key step to understand the neural mechanisms generating behaviors. A newly developed technology called CANE* (Wang lab, manuscript submitted) is able to selectively tag neurons activated during brief ethologically relevant behaviors. This study is focused on social behaviors involving neurons in medial prefrontal cortex (mPFC). Two types of social interaction behaviors are elicited: (1) a social fear behavior in which an intruder male mouse is attacked by an aggressive resident mouse for 10 minutes; and (2) a male-female interaction behavior in which the male is exposed to a female for 30 minutes. mPFC neurons are activated in both behaviors as revealed by anti-Fos staining. We asked whether “social fear” neurons are the same or are distinct from the “mating behavior” neurons in mPFC, and whether they project to the same or different downstream targets. Using the CANE* technology, we expressed GFP in either the “social fear” or the “mating behavior” neurons which allowed us to characterize them. The “projectome” and electrophysiological properties of mPFC “social fear” and “mating” neurons are investigated.


Poster

537. Prefrontal and Striatal Systems: Molecular Mechanisms and Connectivity

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 537.19/BB72

Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant MH45573

NIH Grant NS14944

Title: Projections from the prefrontal cortex and medial and inferior temporal cortices converge in a critical node in the striatum
Abstract: Interactions between the prefrontal cortex (PFC) and the temporal cortex are critical for high-order cognitive functions involving sensory information, memory, and emotion. Both the PFC and medial and inferior temporal cortex (M/ITC) strongly project to the ventral striatum (VS), a major integration site central to making selections and forming associations. However, it is not fully known whether and how PFC and M/ITC projections converge in the striatum. While prefrontal projections to the VS have been mapped in detail previously, the topography of M/ITC projections is only partially known. Here, we undertook a comprehensive mapping of M/ITC projections to the VS and compared this to the topography of PFC-striatal projections. Retrograde injections showed that all parts of the VS receive strong projections from the anterior medial temporal cortex. In contrast, the anterior inferior temporal cortex sends projections primarily to the lateral VS, while the posterior parahippocampal region (pPHR) projects most strongly to the ventral VS. Anterograde injections in the M/ITC confirmed these results and identified at least two types of prefrontal-temporal convergence in the striatum. Projections from the temporal pole (area TG) and the anterior inferior temporal cortex (area TEav) overlap primarily with those from cortical regions involved in emotion processing as follows. Temporal pole projections terminate in the medial and ventral portions of the VS, highly similar to vmPFC (areas 14, 25) projections. They also overlap with OFC (areas 11, 13) and ACC (area 24) projections. Anterior inferior temporal cortex projections terminate in the lateral and mid-dorsal portions of the VS and overlap with those from OFC and ACC. Interestingly, however, projections from the pPHR are split. These terminate in the ventrolateral VS, overlapping with ACC and OFC projections, and also at the dorsal edge of the caudate, where they overlap with dIPFC and premotor projections. Thus, pPHR projections overlap with both emotion and cognitive cortical projections in the striatum. Lastly, the ventral part of the VS appears to be a special region. This region receives input from areas throughout the M/ITC, as well as from the vmPFC, ACC, and OFC. This site of high convergence in the ventral VS may be a critical node that integrates information from diverse functional regions and may be particularly susceptible for disorders. Together, these results indicate that PFC and M/ITC projections have specific convergences in the striatum that involve one or more functional domains. In addition, the ventral VS may contain a critical node of PFC and M/ITC projections.

Disclosures: E. Choi: None. S. Ding: None. G.W. Van Hoesen: None. S.N. Haber: None.

Poster

537. Prefrontal and Striatal Systems: Molecular Mechanisms and Connectivity

Location: Hall A
Title: Projection pattern of the ventrocaudal part of the intralaminar thalamic nucleus to the caudate putamen in the rat brain

Authors: *H. IWAI, E. KURAMOTO, A. YAMANAKA, T. GOTO; Dept. of Oral Anat. and Cell Biol., Kagoshima Univ., Kagoshima, Japan

Abstract: The rat intralaminar thalamic nuclei are enclosed with the internal medullary lamina of the thalamus, and receive input from the brain stem and send output to the cerebral cortex and caudate putamen (CPu). Recently, the medial parabrachial nucleus relaying a visceral sense is reported to project to the ventrocaudal part of the intralaminar thalamic nuclei such as the caudal part of the central medial nucleus and ventral part of the parafascicular thalamic nucleus. Generally, the intralaminar thalamic nuclei project topographically to the CPu, however, it is unclear whether the ventrocaudal part of the intralaminar thalamic nuclei projects topographically to the CPu. Thus, we examined topographical arrangement of neural pathways between the ventrocaudal part of the intralaminar thalamic nuclei and CPu using an anterograde tracer, biotinylated dextran amine, and a retrograde tracer, cholera toxin B subunit. We revealed that the oval paracentral thalamic nucleus mainly projected to the ventrocentral part of the CPu; the ventrolateral part of the parafascicular thalamic nucleus mainly projected to the ventrolateral part of the CPu; and the caudal part of the central medial thalamic nucleus, ventromedial part of the parafascicular thalamic nucleus, and retrogrenoins area mainly projected to the ventromedial part of the CPu. In contrast, the parvicellular part of the posteromedial ventral thalamic nucleus mainly projected to the caudal part of the interstitial nucleus of the posterior limb of the anterior commissure. These results suggest that efferent fibers from the ventral part of the intralaminar thalamic nuclei to the ventral part of the CPu are topographically organized. Because the ventral part of the CPu reportedly regulates food intake, fat intake, and jaw movement, visceral information from the medial parabrachial nucleus is likely to influence feeding behavior in the ventral part of the CPu via the ventrocaudal part of the intralaminar thalamic nuclei.


Poster

537. Prefrontal and Striatal Systems: Molecular Mechanisms and Connectivity

Location: Hall A
Title: A morphological analysis of thalamocortical projections arising from the rat mediodorsal nucleus: A single neuron-tracing study using viral vectors

Authors: *E. KURAMOTO*1, S. PAN2, T. FURUTA2, H. HIOKI2, H. IWAI1, A. YAMANAKA1, S. OHNO1, T. GOTO1, T. KANEKO2

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Abstract: The rat mediodorsal thalamic nucleus (MD) is subdivided into 3 portions, the medial (MDm), central (MDc) and lateral segments (MDl), mainly on the basis of the cytoarchitecture and myeloarchitecture. These 3 MD segments have been reported to receive different inputs from a wide variety of the subcortical structures, and project topographically to the different areas of the prefrontal cortex. However, it is unknown whether the single MD neurons send their axons to only a single cortical area or project widely to multiple areas of the prefrontal cortex. In the present study, the axonal arborization of single MD neurons was examined by a single neuron labeling method with a Sindbis viral vector expressing fluorescent proteins. The location of single MD neurons infected with the Sindbis virus was examined by Nissl-like staining, and further confirmed in immunohistochemistry for calbindin D28k, because the differential distribution of calbindin immunoreactivity is useful for a distinction among the 3 MD segments; calbindin immunoreactivity is more intense in the MDm and MDl than in the MDc. The single MD neurons were completely visualized by immunoperoxidase staining for fluorescent proteins, and their axonal arborization was reconstructed. In the subcortical region, the reconstructed MD neurons emitted axon collaterals always to the thalamic reticular nucleus, and frequently to the striatum. In the cerebral cortex, the main target areas of the MDc, MDm and MDl neurons were lateral orbital, prelimbic/lateral orbital and cingulate/prelimbic areas, respectively. Interestingly, the most MD neurons formed another axonal arborization in the prefrontal cortex. Thus, the single MDl, MDm and MDc neurons innervated multi-areas with a considerable overlap, indicating that the 3 pieces of information conveyed via the 3 MD segments would be integrated in the thalamocortical projection pathway. On the basis of their different cortical projections, thalamic neurons are divided into two types: core-type and matrix-type neurons project to the middle layers and superficial layer, respectively. Since the axon fibers of most MD neurons were abundantly distributed in layers 2-5, MD neurons were classified to core-type, and their axon fibers might target to the basal dendrites of layers 2-5 pyramidal neurons. These results suggest
that even when a small number of MD neurons are activated, a large number of Layers 2-5 pyramidal neurons in multi-areas would be simultaneously activated, and that the coordinated activation of multi-areas may be lead to an appropriate execution of prefrontal functions, such as behavioral flexibility, recognition memory and attention.


**Poster**

538. Motivation and Emotion: Reward I

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 538.01/BB75

**Topic:** F.03. Motivation and Emotion

**Support:** Howard Hughes Medical Institute

David Rockefeller Fellowship

**Title:** Cell-type-specific control of innate behaviors by the dorsal raphe nucleus

**Authors:** *A. R. NECTOW¹, B. FIELD², J. M. FRIEDMAN²;
¹Dept. of Mol. Genet., Rockefeller Univ., New York, NY; ²Mol. Genet., Rockefeller University/HHMI, New York, NY

**Abstract:** The dorsal raphe nucleus (DRN) has been implicated in the modulation of a number of critical, survival-related functions such as reward, food intake, and locomotion. In addition to being the single largest serotonergic nucleus in the mammalian brain, the dorsal raphe also houses a number of other cell types such as glutamatergic neurons (expressing VGluT3) and GABAergic neurons (expressing Vgat). In the current studies, we used a combination of molecular profiling, optogenetics, and viral tracing to establish the network function of the dorsal raphe nucleus in controlling survival-related behaviors. These studies have elucidated novel, unexpected roles for both the glutamatergic and GABAergic neurons in bidirectional modulation of food intake, as well as differential contributions to locomotor function. Together, these data demonstrate a significant, previously unappreciated role of the dorsal raphe’s non-serotonergic neurons in controlling diverse innate behaviors.

**Disclosures:**  A.R. Nectow: None. B. Field: None. J.M. Friedman: None.
**Poster**

538. **Motivation and Emotion: Reward I**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 538.02/BB76

**Topic:** F.03. Motivation and Emotion

**Title:** The role of median raphe GABA and glutamate neurons in reward

**Authors:** *A. TAN*, S. IKEMOTO;
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**Abstract:** Previous pharmacological research has shown that rats will self-administer GABA receptor agonists, AMPA receptor antagonists, and NMDA receptor antagonists into the median raphe region (MR). However, there are diverse populations of neurons serotoninergic, glutamatergic, and GABAergic in the MR, and the roles they play in reward are unclear. To resolve this issue, the present study utilized optogenetics to selectively excite or inhibit particular populations of MR neurons. Wild-type C57 mice received a viral vector encoding halorhodopsin (NpHR) in the MR, and learned to self-administer photostimulation, suggesting that net inhibition of MR neurons is rewarding. Transgenic vGat-Cre mice received NpHR in the MR, resulting in selective expression of the opsin in GABA neurons. These mice learned to self-administer photostimulation at a greater level than C57 mice, suggesting that inhibition of GABA neurons is one part of the circuitry underlying MR reward. Transgenic vGluT3-Cre mice received channelrhodopsin (ChR2) in the MR, resulting in selective expression of the opsin in glutamate neurons. These mice also learned to self-administer photostimulation at a greater level than C57 mice, suggesting that excitation of glutamate neurons is another part of the circuitry underlying MR reward. Taken together, inhibition of MR GABA neurons may disinhibit MR glutamate projection neurons, which activate global reward circuits. The MR may be another important region to study in order to understand the neurobiology of affect and addiction.

**Disclosures:** A. Tan: None. S. Ikemoto: None.
Abstract: The orbitofrontal cortex (OFC) is important for the cognitive processes of learning and decision making. Previous recordings have revealed that OFC neurons encode predictions of reward outcomes. The OFC is interconnected with the dorsal raphe nucleus (DRN), which is a major serotonin center of the brain. Recent studies have provided increasing evidence that the DRN encodes reward signals. However, it remains unclear how the activity of DRN neurons affects the prospective reward coding of OFC neurons. By combining single-unit recordings from the OFC and optogenetic activation of the DRN in behaving mice, we found that DRN stimulation is sufficient to organize and modulate the anticipatory responses of OFC neurons. During Pavlovian conditioning tasks for mice, odorant cues were associated with the delayed delivery of natural rewards of sucrose solution or DRN stimulation. After training, OFC neurons exhibited prospective responses to the sucrose solution. More importantly, the coupling of an odorant with delayed DRN stimulation resulted in tonic excitation or inhibition of OFC neurons during the delay period. The intensity of the prospective responses was affected by the frequency and duration of DRN stimulation. Additionally, DRN stimulation bidirectionally modulated the prospective responses to natural rewards. These experiments indicate that signals from the DRN are incorporated into the brain reward system to shape the cortical prospective coding of rewards.

Abstract: Motivation is a complex process that involves appetitive and consummatory components. A number of neurotransmitters and neural circuits have been shown to be involved in this process. Dopamine (DA) signaling is often suggested to mediate reward, but the precise nature of this involvement seems to be more specific; DA receptor antagonists decrease operant responding for food reinforcers, but leave consummatory and hedonic responses relatively unchanged. Opioid signaling mediates consummatory behavior, an effect suggested to be associated with altered palatability and hedonic responses to food reinforcers. Opioid receptor antagonists also decrease operant responding for food on progressive (PR) ratio schedules, indicating that this system may also be involved in appetitive, instrumental behavior. The present study examined the role of opioid and dopamine receptors in food reinforcement using an effort-related decision-making task (i.e., a progressive ratio/chow feeding task). With this task, rats can choose between working for a preferred food (high-carbohydrate banana-flavored sucrose pellets) by lever pressing on a PR schedule vs. obtaining less preferred laboratory chow that is freely available concurrently in the chamber. Haloperidol (0, 0.05, 0.1 mg/kg; i.p.) decreased the breakpoint (highest PR achieved), lever presses, and number of reinforcers earned, and increased chow intake (an effect found with 0.05 and 0.1 mg/kg). By contrast, naloxone (0, 1.5, 3 mg/kg; i.p.) decreased breakpoint and number of reinforcers earned (using 3, but not 1.5 mg/kg) but had no effect on chow consumption. A preference test measured the relative intake of both foods (banana pellets vs. chow) under effort-free conditions. Haloperidol (0.1 mg/kg) produced a decrease in intake of both foods but did not affect preference. Opioid receptor blockade (naloxone 3 mg/kg) selectively reduced banana pellet intake but had no effect on chow. The present findings support evidence suggesting that DA signaling mediates activational components of appetitive motivation, particularly those related to exertion of effort, while leaving some aspects of consummatory behavior (i.e., palatability or food preference) unaffected. Opioid signaling appears to selectively affect intake of relatively preferred foods. Based on previous literature, we hypothesize that a naloxone-induced decrease in palatability for preferred food in our experiment resulted in reduced willingness to exert effort. Our results argue against a general suppression of appetite by either compound as appetite suppressants have been previously shown to alter intake of both types of food regardless of the task.

Title: Low frequency rTMS to monkey STS moderates neuronal sensitivity to social reward

Authors: *A. UTEVSKY\(^1\), M. L. PLATT\(^2\);
\(^1\)Ctr. for Cognitive Neurosci., \(^2\)Dept. of Neurobio., Duke Univ., Durham, NC

Abstract: The superior temporal sulcus (STS) region of cortex contributes to various aspects of social cognition, including face perception, biological motion detection, joint attention, empathy, and theory-of-mind. Recent brain imaging studies have also implicated this area in social decision-making. The precise neuronal mechanisms mediating STS contributions to social decisions remain unknown. To answer this question, we used a combination of repetitive transcranial magnetic stimulation (rTMS) to STS and simultaneous neuronal recordings in the same area in monkeys performing a social reward-allocation task (Chang et al., 2011, 2012, 2013). Monkeys chose to allocate juice rewards to self, another monkey, both, or no one. Consistent with our prior published studies, monkeys preferred to reward the recipient monkey over no one, but preferred to reward self over both monkeys. Single and multi units in the middle STS responded strongly during the choice commitment and reward-outcome phases of the task. Moreover, firing rates varied systematically with the social context of decision, responding most strongly to “both” and “self” trials and significantly less to “other” and “none” trials. Importantly, firing rates were significantly higher when monkeys chose “other” over “none,” and these preferences were magnified in a second condition that cued different magnitudes of reward. Critically, 10 minutes of low-frequency (1Hz) rTMS - a stimulation regime thought to suppress neuronal activity - to the STS abolished social preferences expressed behaviorally and simultaneous neurophysiological distinctions between social reward outcomes. Sham stimulation had no impact on social preferences or neurophysiological activity. Together, these findings suggest STS neurons signal predicted and experienced social reward outcomes and that these...
signals contribute directly to social decisions. Our data also demonstrate that low-frequency rTMS impairs behavior by disrupting neurophysiological activity at the site of stimulation.

Disclosures: A. Utevsky: None. M.L. Platt: None.

Poster

538. Motivation and Emotion: Reward I

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 538.06/BB80

Topic: F.03. Motivation and Emotion

Support: NIH-NCRR (2G12-RR003051)
NIMHD (8G12-MD007600)
Conte Center Grant (P50 MH086400)
MBRS-RISE-MSC (R25-GM061838)

Title: Low-frequency deep brain stimulation of the ventral striatum facilitates the extinction of morphine place preference

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Abstract: Recently, research in humans and rodents has indicated that DBS of the ventral striatum (VS) may be an effective treatment for drug addiction (Luigjes et al., 2012). However, we recently found in rats that high-frequency DBS (HF-DBS) of the VS impaired extinction of morphine-induced conditioned place preference (CPP; Martinez-Rivera et al., SFN 2013). Furthermore, low-frequency DBS (LF-DBS) has also been suggested as a treatment of neuropsychiatric disorders in humans (Hernando et al., 2008). In rats, LF-DBS of the VS attenuated cocaine sensitization (Creed et al., 2015) and relapse (Hamilton et al., 2014). However, no studies have applied LF-DBS of the VS during the extinction of drug-associated memories. In this study, we examined whether LF-DBS of the VS facilitates the extinction of morphine-CPP. Rats were implanted with DBS electrodes in the VS and conditioned to prefer a side paired with morphine. Subsequently, rats expressing morphine-CPP received extinction
sessions, together with 60 min of LF-DBS (20 Hz) or sham stimulation. Our results showed that LF-DBS of the VS had no effect during extinction training, but strengthened extinction memory when tested 2 days (p=0.005) or 9 days (p=0.04) after stimulation was turned off. In addition, LF-DBS increased e-Fos immunolabeling in the infralimbic cortex (p=0.03) and medial portion of central amygdala (p=0.04), key regions in the extinction of drug seeking behaviors (Gass and Chandler, 2013). Our results support the idea that LF-DBS (rather than HF-DBS) of the VS represents a possible therapy for treatment-resistant opioid addicted patients, who undergo extinction-based therapies.


Poster

538. Motivation and Emotion: Reward I

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 538.07/BB81

Topic: F.03. Motivation and Emotion

Support: NHMRC Grant 628495

Title: Intact anterior insular response to punishment magnitude, despite dACC related error-learning deficits in heroin dependent participants

Authors: *D. J. UPTON, D. A. O'CONNOR, J. MOORE, K. P. CHARLES-WALSH, S. ROSSITER, R. HESTER;
Sch. of Psychological Sci., The Univ. of Melbourne, Melbourne, Australia

Abstract: Deficits in error processing and error-related learning are well documented in substance dependent individuals (SDIs). While evidence suggests the dorsal anterior cingulate cortex (dACC) is involved in learning from errors, other regions such as the anterior insula (AI) appear to mediate behavioural adjustments in response to the level of punishment an error may produce. The aim of this study was to examine the roles of dACC and AI in error-related learning in SDIs. A group of opiate dependent participants (N=22) and non-dependent controls (n=20) performed a paired-associate recall task that delivered a monetary penalty for incorrect responses (5c, 50c). dACC and AI BOLD responses following error feedback and target encoding were used to predict subsequent recall performance in both groups. dACC activity during target encoding in the control group, but not in the heroin dependent group, was
associated with subsequent recall performance following an incorrect response; however activity in this region was not sensitive to punishment magnitude. In contrast, AI activity was higher in both groups following the larger penalty, and higher AI activity during penalty feedback was associated subsequent recall performance. These data confirm previous findings in SDIs implicating the dACC in error-learning deficits, but suggest that an AI mediated punishment response is intact.


Poster

538. Motivation and Emotion: Reward I

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 538.08/BB82

Topic: F.03. Motivation and Emotion

Support: This study was supported by the grant SONATA BIS 2012/07/E/NZ3/01785 from the Polish National Science Centre.

Title: A new model to study reward discounting in mice living in groups

Authors: *L. SZUMIEC, J. RODRIGUEZ PARKITNA;
Inst. of Pharmacol. PAS, Krakow, Poland

Abstract: Here we present a new model of testing reward discounting in group-housed mice. A cohort of 12-16 mice (C57-BL/6J females) is implanted subcutaneously with radiofrequency identification chips and placed in a cage equipped with sensors for automatic tracking (IntelliCage Plus). Each of the cage’s corners has 2 drinking bottles, accessed through a slight chamber that allows only one mouse inside and it can be individually monitored and restricted to model the effects of reward discounting. We have tested three types of discounting related to the delay, probability or risk of a reward. The effect of the delay was assessed by first allowing mice free access to water or 0.1% (w/v) saccharin solution and then progressively increasing the delay till gate blocking the saccharin bottle raised. In line with expectations, while mice initially showed 95.4% preference for saccharine it decreased to 50% at 17s delay and finally to 12% at 55s delay. In case of the probability discounting model we have tested a choice between 3 types of reward (saccharin 0.1% or 0.01% and water). Mice were able to access saccharin with a probability of 0.25 or 0.75 and had normal access to water. Preference of the 0.1% saccharin with at 0.75 probability was 69%, compared to 9% preference of the bottle with 0.01% saccharin
at 0.75 probability. In case of the risk discounting model we have tested the effect a 0.25 probability of punishment (an airpuff to the back) on preference of 0.1% saccharin. As expected, while preference of saccharin was initially 95%, the risk of punishment decreased it to 39%. To validate the model we tested the effects of an irreversible inhibitor of the monoamine oxidase - tranylcypromine on delay discounting. In line with expectations, the drug-induced increase in monoamine levels caused acceleration of delay discounting. Observed preference at 17s delay was 4% in the drug-treated group compared to 51% in controls. The main advantages of the new model are the ability to test behaviour in the home cage, in group-housed mice, during natural activity cycles, no interaction with the experimenter and without food deprivation. We hope this model will be useful to study the effects of pharmacological treatments or genetic on modifications on reward discounting.

**Disclosures:** L. Szumiec: None. J. Rodriguez Parkitna: None.

**Poster**

**538. Motivation and Emotion: Reward I**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program/#Poster#:** 538.09/BB83

**Topic:** F.03. Motivation and Emotion

**Support:** MH048404

**Title:** Ventral tegmental area and substantia nigra pars compacta exhibit similar neural responses to reward-related cues and events

**Authors:** *M. A. WEGENER¹, B. MOGHADDAM²;*  
¹Ctr. For Neurosci. Univ. of Pittsburgh, Pittsburgh, PA; ²Neurosci., Univ. of Pittsburgh, Pittsburgh, PA

**Abstract:** Research investigating motivation and psychiatric disorders has identified dopamine neurons as a crucial part of the mechanism behind reward-mediated behavior. Much of this work has revolved around the limbic system in rodents by closely examining the activity of dopamine neurons in the ventral tegmental area (VTA) and their influence on the nucleus accumbens. Recently, research has recognized the potential contribution of the substantia nigra pars compacta (SNCs), a separate dopaminergic region with distinct efferent and afferent projections, toward guiding motivated behavior. However, little is known about how SNC neurons process reward-related events. Here we aimed to directly compare the response pattern of VTA and SNC neurons within the same animal during reward-mediated instrumental behavior. Adult rats were
implanted bilaterally with microelectrode arrays to conduct single-unit recordings in the VTA and SNC during task acquisition and maintenance. In this task, rats learned to execute a naturalistic nose poke during presentation of a cue to receive a single sugar pellet. Electrophysiological data from these animals suggest that neurons within the VTA and SNC exhibit similar phasic responses to cues predicting the availability of reward and reward delivery. This parallel population activity persists across all sessions of the task. Future analyses will compare population and single unit activity within the same animal and will seek to identify any subpopulations within regions that encode specific task-related events. These data so far suggest that reward responsiveness of VTA neurons generalizes to the SNC.

Disclosures: M.A. Wegener: None. B. Moghaddam: None.

Poster

538. Motivation and Emotion: Reward I

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#/Poster#: 538.10/BB84

Topic: F.03. Motivation and Emotion

Title: Physical activity deficits in obese animals are linked to dysfunction of striatal D2-receptors

Authors: *D. M. FRIEND1,3, K. DEVARAKONDA1,2, V. ALVAREZ4,2, K. HALL3,2, A. KRAVITZ3,2;

1NIH, Arlington, VA; 2NIH, Bethesda, MD; 3Natl. Inst. of Diabetes and Digestive and Kidney Dis., Bethesda, MD; 4Natl. Inst. on Alcohol Abuse and Alcoholism, Rockville, MD

Abstract: Obese individuals report low levels of physical activity, despite knowledge that increased physical activity improves cardiovascular health and may lead to weight loss. However, it is not clear why obese individuals have low levels of physical activity. Based on clinical studies demonstrating impairments in dopamine D2 receptor (D2R) function in the striatum of obese individuals and data heavily implicating striatal circuits in physical activity, we hypothesized that 1) impaired striatal D2R function alters basal ganglia circuitry, which decreases physical activity in obese animals; and 2) this decrease in physical activity contributes to further weight gain. To test this hypothesis, we fed mice a high-fat diet and found that striatal D2R function decreased as the animals gained weight. In parallel with decreased D2R function was a reduction in physical activity. To more explicitly test whether eliminating the D2R in striatal neurons was sufficient to decrease physical activity, we selectively eliminated D2Rs from indirect pathway striatal neurons using a novel conditional knock out strategy (Drd2-KO mice).
Drd2-KO mice were hypoactive relative to wild-type (WT) mice, confirming our first prediction that decreases in striatal D2Rs are sufficient to decrease physical activity. We examined our second prediction by measuring weight gain in Drd2-KO mice and WT mice on a high fat diet. However, despite having decreased physical activity, Drd2-KO gained weight at the same rate as WT mice. We conclude that high-fat diet reduces striatal D2Rs, causing low physical activity levels in obese animals; however, this decrease in physical activity does not contribute to further weight gain but improves metabolic health. We are currently performing experiments using novel chemogenetic techniques to specifically manipulate activity of D2R-expressing cells in obese animals to restore physical activity. These findings help explain at a biological level why obese people have low rates of exercise, while also addressing the efficacy of exercise as an intervention in human obesity.


Poster
538. Motivation and Emotion: Reward I

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 538.11/BB85

Topic: F.03. Motivation and Emotion

Support: NIDDK Intramural Research Program

Title: Food intake better predicts weight gain than physical activity and D2 receptor availability

Authors: *K. DEVARAKONDA, D. M. FRIEND, J. GUO, K. D. HALL, A. V. KRAVITZ; NIDDK, Bethesda, MD

Abstract: Nearly two-thirds of adults in the United States are overweight or obese. Physicians generally advise patients to “eat less” and “move more” to combat weight gain and metabolic syndrome. However, it is unclear why individuals consume more calories than they burn. The striatal dopamine system has been linked to compulsive food intake and obesity, as well as the regulation of movement. To better understand the role of diet, physical activity, and dopaminergic function in the development of obesity, we measured food intake, physical activity, energy expenditure, and dopamine D2 receptor (D2R) expression prior to placing male C57BL/6 mice on a long-term high-fat diet alongside weight-matched controls that remained on chow. In a multiple regression analysis, we found that caloric intake was a better predictor of weight gain than physical activity, energy expenditure, or D2R binding. Our data contradicts
previous findings that striatal D2R availability predicts future weight gain in humans and suggests that changes in diet play a larger role in weight gain than changes in physical activity.


Poster

538. Motivation and Emotion: Reward I

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#/Poster#: 538.12/BB86

Topic: F.03. Motivation and Emotion

Support: NSERC grant RGPIN 308-11

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Concordia University Research Chair

Title: Modulation of reward seeking by changes in energy balance: a 3D perspective

Authors: *S. NOLAN-POUPART*, K. CONOVER2, P. SHIZGAL2;
2Ctr. for Studies in Behavioural Neurobio., 1Concordia Univ., Montreal, QC, Canada

Abstract: The relationship between peripheral energy balance and reward seeking has long been studied in rats working for rewarding electrical stimulation of the lateral hypothalamus (LH). When the electrode tip is dorsolateral to the fornix, decreases in body mass potentiate operant performance. When the electrode is located outside this area, performance is typically unaffected by energy manipulation, suggesting that the substrate that supports brain stimulation reward (BSR) is functionally heterogeneous. Prior studies were carried out using two-dimensional (2D) measurements: the vigor of performance was measured as a function of stimulation strength, and the effect of energy manipulation was quantified by the stimulation strength required to produce half-maximal performance. The interpretation of such findings has been questioned by the introduction of a three-dimensional (3D) method that measures reward seeking as a function of both the strength and cost of reward. This new method eliminates an ambiguity inherent in 2D measurements while providing information about the stage(s) of neural processing at which energy balance affects reward seeking. We reexamined the effect of energy manipulation on BSR using the 3D approach. To this end, 10 male Long-Evans rats were trained to hold down a lever so as to earn trains of LH stimulation. A stimulation train was delivered when the cumulative hold time reached a criterion that defined the cost of BSR (the “price”). Stimulation
strength was set by the frequency with which current pulses were delivered within 0.5 s fixed-duration trains. A plot of lever hold time as a function of pulse frequency and price yields a 3D surface resembling the corner of a plateau (the “reward mountain”). Displacement of the mountain along the pulse-frequency axis reflects changes in early reward processing consistent with altered reward sensitivity, whereas displacement along the price axis reflects changes at later stages, such as alteration of the rat’s proclivity to invest effort in reward seeking. Following baseline testing, the rats were food restricted until their weight reached 75% of the baseline mean. This caused a multiplicity of changes, including shifts in either direction along the pulse-frequency axis (reflecting changes in reward sensitivity) and along the price axis (consistent with changes in the proclivity to invest effort in reward seeking). Thus, application of the 3D method has shed further light on the heterogeneous effects of energy manipulations on reward seeking. Moreover, reliable changes were seen more frequently than in prior 2D studies, thus attesting to the increased power of the 3D approach.

Disclosures: S. Nolan-Poupart: None. K. Conover: None. P. Shizgal: None.

Poster

538. Motivation and Emotion: Reward I

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Program#/Poster#: 538.13/BB87

Topic: F.03. Motivation and Emotion

Title: Brain areas involved in detecting valuable objects: a functional MRI study in macaques

Authors: *A. GHAZIZADEH1, W. GRIGGS1, D. A. LEOPOLD2, O. HIKOSAKA1;
1LSR NIH, Bethesda, MD; 2NIMH, Bethesda, MD

Abstract: Reward attracts attention and gaze. When humans and monkeys experience visual objects with large rewards repeatedly, their attention and gaze are attracted by the objects even if reward is no longer given. The basal ganglia, especially their posterior part, play an important role in such learned attention/gaze orientation (Hikosaka et al 2014). However, other brain areas may also be involved in learned attention/gaze. To address this issue, we performed functional magnetic resonance imaging (fMRI) using monocrystalline iron oxide nanoparticles (MION) in two rhesus monkeys. Before the fMRI scans, the monkeys were shown many computer-generated fractals (n>80), each of which was rewarded consistently with either a large or small juice amounts over >10 days. These objects became ‘good’ (automatically attracting gaze) or ‘bad’ (rejecting gaze). During the fMRI scan, monkeys fixated centrally while passively viewing fractals. In one block of trials, all fractals were either good or bad and were presented in either
the left or right hemifield (2 value x 2 hemifields = 4 block types). Each of the task blocks was preceded by a rest block in which monkeys fixated centrally with no objects presented. To keep animals motivated, occasional rewards were given in all blocks with the same random distribution. Comparison of good vs bad blocks revealed stronger activations by good than bad objects in temporal cortical areas (V4, TEO, TE), lateral prefrontal cortex (lPFC), and lateral intraparietal area (LIP). Some value coding was found in subcortical areas, including posterior-ventral putamen, caudate tail, and amygdala. Importantly, many of these value sensitive regions also showed a laterality effect, responding more strongly to contralateral than ipsilateral objects. These results show that valuable objects receive enhanced processing in temporal cortical areas involved in object recognition and in areas involved in attention such as LIP and lPFC. This enhanced processing can explain the attentional capture by valuable objects following long-term reward association. Such a visual skill and its underlying brain mechanisms would be important for reward-maximization and survival.


Poster

538. Motivation and Emotion: Reward I

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 538.14/BB88

Topic: F.03. Motivation and Emotion

Support: R00-MH099093

R01-MH095894

W81XWH-11-1-0584

Title: Pupillary correlates of vicarious reward in rhesus macaques

Authors: *J. A. JOINER¹, N. A. FAGAN¹, M. L. PLATT², S. W. C. CHANG¹³;
¹Psychology, Yale Univ., New Haven, CT; ²Neurobio., Duke Univ., Durham, NC; ³Neurobio., Yale Univ. Sch. of Med., New Haven, CT

Abstract: Pupil diameter is thought to reflect the internal states of animals like motivation and emotion. In the motivational domain, pupil diameter scales with the magnitude of experienced reward (1). In the social domain, reward experienced vicariously (i.e., through social observation) powerfully shapes behaviors, suggesting that there is a close relationship between
vicarious reward and internal state. However, it remains unknown whether pupil diameter also reflects vicarious reward. In order to determine the link between pupillary response and vicarious reward, we examined pupil dilation during a social decision-making task in rhesus macaques (*Macaca mulatta*). In this task, actor monkeys prefer to deliver juice reward to themselves (Self) over both themselves and a recipient monkey (Both) on one trial type but prefer to deliver juice to the recipient (Other) over no one (Neither) on the other trial type, suggesting a context-specific role of vicarious reinforcement (2). We quantified changes in pupil diameter using a generalized linear model (GLM) for the different reward outcomes (Self, Both, Other, Neither) while controlling for any changes due to saccades directed at the recipient by the actors following reward delivery. The pupil diameter for each outcome differed and systematically tracked the subjective decision preferences, correlating inversely with reaction times of choosing each reward option. As expected, the pupil size was larger when actors received reward compared to when they did not. Notably, pupil size was larger for Self compared to Both rewards despite the fact that either option resulted in the same amount of reward to the actors. Crucially, pupil size was also significantly larger for Other than Neither rewards even though either option always resulted in no reward to the actors, suggesting that vicarious reward is reflected by changes in pupil size. By contrast, on non-social trials, in which the recipient was replaced by a juice collection bottle, the pupil size simply differentiated actors’ rewarded (Self, Both) and unrewarded (Other, Neither) outcomes but did not differentiate between Other and Neither nor Self and Both. Our results suggest that vicarious reward corresponds to a change in a motivational state and emphasize a potentially important role of the autonomic nervous system in mediating social interactions. References 1. Kennerley SW & Wallis JD (2009). Reward-dependent modulation of working memory in lateral prefrontal cortex. J Neurosci 29, 3259-70. 2. Chang SWC, Winecoff AA & Platt ML (2011). Vicarious Reinforcement in Rhesus Macaques (*Macaca Mulatta*). Front. Neurosci. 5.


Poster

538. Motivation and Emotion: Reward I

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program# Poster#: 538.15/BB89

Topic: F.03. Motivation and Emotion

Support: SIMONS FOUNDATION

R00-MH099093
Title: Seeing eye-to-eye: live gaze interactions in pairs of rhesus macaques robustly capture dominance behavior

Authors: *O. DAL MONTE*¹, M. PIVA², J. A. JOINER¹, W. PACK¹, A. C. NAIR¹, S. W. C. CHANG¹;²
¹Dept. of Psychology, Yale Univ., New Haven, CT; ²Neurobio., Yale Univ. Sch. of Med., New Haven, CT

Abstract: The most important feature of social interaction is the dynamic exchange between two individuals. In humans and non-human primates, gaze behavior is an important component of social communication and interaction that allows identification of group members and social status, interpretation of facial signals, and formulation of appropriate behavioral responses. To study real-time social interaction, we developed a novel paradigm to assess live free viewing in pairs of rhesus macaques. In this paradigm, two monkeys were placed in front of each other while eye positions were recorded from the two animals simultaneously. To compare our new paradigm with more traditional experimental setups, we also measured eye gaze patterns directed toward static images of the paired monkey. Each monkey was additionally tested for dominance with a food-grabbing task and a social encounter task, in which head and body orientation were measured for each monkey with respect to the other. We examined differences in dynamic exploration patterns including mutual gaze between dominant and subordinate monkeys. As expected, the dominant animal looked at the face and eye region of the other more frequently and for longer, whereas the subordinate animal actively avoided the dominant by looking away from his face or even closing his eyes. These results recapitulate the two independently measured food-grabbing and social encounter tasks, in which subordinate monkeys grab less food and orient their head and body away from dominant. Of particular interest, monkeys explored each other’s faces for a shorter duration and less frequently in the live context compared to the static context. Strikingly, differences in gaze behavior between dominant and subordinate monkeys were less pronounced when exploring static images of the monkeys as opposed to interacting with other animals. Given the difference in gaze patterns between live and static contexts, increased ecological validity in the live gaze interaction setting could elicit meaningful differences in interactive behaviors that might not be captured in more traditional experimental settings. Our paradigm allows for analyzing real-time gaze interaction between two individuals with a level of granularity that is not possible in other social interaction paradigms. This methodology could be implemented in both experimental and clinical contexts to evaluate nuanced and dynamic social behaviors.


Poster

538. Motivation and Emotion: Reward I
Title: Counterbalancing prosocial decisions across egocentric and allocentric reward contexts in rhesus macaques

Authors: *W. D. PACK*¹, J. A. JOINER¹, S. W. C. CHANG¹²;
¹Psychology, Yale Univ., New Haven, CT; ²Neurobio., Yale Univ. Sch. of Med., New Haven, CT

Abstract: A reward outcome experienced by others could be vicariously rewarding or perceived as competitive and even aversive depending on various contexts, promoting either prosocial or antisocial behavior. Rhesus macaques (*Macaca mulatta*) show such context-dependent social preferences when making decisions impacting reward outcome of self and other. In a dictator game, an actor monkey prefers to deliver juice reward to a recipient over no one in one (allocentric) context but prefers to deliver juice reward to himself over simultaneously to both himself and the recipient in the other (egocentric) context. One possible explanation for the antisocial preference in the egocentric context is that consuming rewards simultaneously with a conspecific instigates a competitive process. To test this idea, we examined social behaviors of rhesus macaques in a modified dictator game in which the relative timing between juice rewards delivered to the actor and the recipient was systematically varied for shared juice rewards. Relative to simultaneous reward delivery, the amount of competition should be lower when the actor receives the reward before the recipient, but higher in the reverse order. Consistent with this, when the actor’s reward was slightly delayed relative to the recipient’s reward, the frequency of sharing greatly decreased relative to the no-delay condition (simultaneous reward onset). In contrast, when the actor’s reward was followed by the recipient’s reward, the frequency of sharing increased relative to the no-delay condition. These observations suggest that competition may be driving the antisocial preference in the egocentric context. Most strikingly, we observed that the actor actively counterbalances his decision preferences across the egocentric and allocentric contexts in order to compensate the timing-induced increase or decrease in sharing in the egocentric context by decreasing or increasing reward donation in the allocentric context, respectively. These behaviors suggest that the actor keeps tally on the desired reward intake of the recipient across trials. Taken together, our results paint a complex picture of social preference as the intersection of two different processes, competition and vicarious
reinforcement. Furthermore, the counterbalancing behavior of prosocial and antisocial decisions across the two fundamentally different reward contexts reveals advanced social cognition in rhesus macaques.

**Disclosures:** W.D. Pack: None. J.A. Joiner: None. S.W.C. Chang: None.

**Poster**

**538. Motivation and Emotion: Reward I**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 538.17/BB91

**Topic:** F.03. Motivation and Emotion

**Title:** Impulsivity and accubofrontal white matter integrity

**Authors:** *T. IKUTA*¹, K. H. KARLSGODT²,³,⁴;
¹Univ. of Mississippi, University, MS; ²Zucker Hillside Hosp., Glen Oaks, NY; ³Feinstein Inst. for Med. Res., Manhasset, NY; ⁴Hofstra North Shore-LIJ Sch. of Med., Hempstead, NY

**Abstract:** Glutaminergic innervation from the prefrontal cortex (PFC) to the nucleus (NAcc), also known as the accubofrontal tract, has been shown to be a part of the circuitry for decision making. However, this mono-synaptic innervation has not well been studied in human samples until recently (Karlsgodt et al, forthcoming). Here we aimed to examine the association of the white matter integrity of the tract and impulsivity in decision making. Using Diffusion Tensor Imaging (DTI) samples from Nathan Kline Institute / Rockland sample, the accubofrontal tracts were estimated by probabilistic tractography for 145 individuals who are 21 years or older (Fig 1). Fractional anisotropy (FA) was extracted for the bilateral accubofrontal tracts in each individual. To estimate the impulsivity of each individual, the UPPS Impulsive Behavior Scale (Whiteside and Lynam, 2001) total score was used from the NKI-Rockland sample. The association between Impulsivity and FA were tested along with age and sex as covariates. The accubofrontal tract white matter integrity showed significantly positive association (p=0.037, Fig 2). The current preliminary results suggest that the strength of the anatomical connectivity in accubofrontal tract predicts impulsivity. Not only does the current finding re-affirms the role of the frontal dopaminergic circuitry to impulsivity, but also shows that accubofrontal connectivity at least partly accounts for the variance in impulsivity across individuals.
Disclosures:  T. Ikuta: None. K.H. Karlsgodt: None.

Poster

538. Motivation and Emotion: Reward I

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 538.18/BB92

Topic: F.03. Motivation and Emotion
Support: DFG PE 1627/4-1

Title: Parcellating the effects of medial orbitofrontal cortex lesions on value-based decision-making

Authors: *J. PETERS*¹, M. D'ESPOSITO²;
¹Dept. of Systems Neuroscience, Univ. Medical-Center Hamburg-Eppendorf, Hamburg, Germany; ²Helen Wills Neurosci. Institute, Univ. of California, Berkeley, CA

Abstract: Parcellating the effects of medial orbitofrontal cortex lesions on temporal discounting. Jan Peters & Mark D'Esposito Background. Damage to orbitofrontal cortex (OFC) increases impulsivity (Bechara et al., 1997), i.e. action with little forethought or deliberation. One way to measure impulsive choice is via temporal discounting tasks, where participants choose between smaller-sooner (SS) and larger-later (LL) rewards. OFC damage leads to an increase in SS choices (Sellitto et al., 2010), i.e. steeper temporal discounting, similar to addiction (Bickel et al., 2014). By what mechanism might OFC damage increase temporal discounting? We examined two possibilities. First, OFC lesions lead to more random decisions (Fellows & Farah, 2007; Henrio-Barghava et al., 2012). This can be erroneously taken to reflect increases in discounting (Franco-Watkins et al., 2006). Second, OFC is part of a network involved in representing reward values (Bartra et al., 2013). Damage to OFC might thus impair LL reward representations, thereby increasing SS choices. Methods. Traumatic brain injury patients with MRI-confirmed damage to OFC (n=9, maximum lesion overlap in centro-medial OFC) and matched controls (n=13) performed a temporal discounting task where one condition involved immediate SS rewards (Would you prefer $10 now or $20 in 60 days?). Another condition involved only delayed rewards (Would you prefer $10 in 30 days or $20 in 90 days?). In a simple rating task (Figner et al., 2010) participants rated the attractiveness of different individual reward options (e.g. 40$ in 10 days, 10$ tomorrow) on a visual-analogue scale. The slope of the amount-attractiveness relation was taken as a measure of individual reward sensitivity. Results. Patients tended to discount steeper (main effect group: p=.08), in particular when an immediate reward was available (group x condition interaction: p=.025). Choices were numerically more random in OFC patients but this effect was non-significant and unrelated to temporal discounting. In contrast, reward sensitivity from the rating task correlated with temporal discounting (r=−.55, p=.0075) and this effect was qualitatively similar in patients (r=−.38, p=.32) and controls (r=−.57, p=.04). Reward sensitivity was significantly reduced in OFC patients (p=.036). Discussion. Our findings extend previous results regarding OFC lesion effects on temporal discounting (Sellitto et al., 2010): reward magnitude sensitivity was correlated with discounting, and impaired in OFC patients. Increased temporal discounting following OFC damage may thus be due to a disruption of reward valuation processes.

Disclosures: J. Peters: None. M. D'Esposito: None.
Title: The neuronal population in monkey ventral striatum encodes both reward size and delay to obtain it

Authors: *R. FALCONE, D. WEINTRAUB, G. CHEN, B. RICHMOND; NIMH, Bethesda, MD

Abstract: Two factors that influence the subjective value of a reward are its size and the amount of time that passes before the reward’s delivery. After bilateral damage to the ventral striatum animals become appear to lose motivation. We recorded neuronal responses in the ventral striatum in two monkeys while they performed a task in which we offered 9 combinations of reward by mixing 3 sizes (2, 4 or 6 drops of water) and 3 delays (1, 5 or 10s). A cue indicating the combination being offered was presented throughout the trials. There were two response periods. On appearance of a yellow dot, the monkeys could refuse the offer by releasing a bar immediately or accept by releasing when a purple dot appeared. If a purple dot appeared, the monkeys could accept by releasing immediately or refuse by waiting for the yellow dot. The probability of accepting the offered reward was highest for the largest reward with the shortest delay, and became progressively smaller as the reward became smaller and/or the delay became longer. The probability of accepting was modeled using a logistic regression model with two continuous explanatory variables, reward size and delay. We analyzed the firing rate of the ventral striatum neurons in the initial period after the cue appeared on the screen, but before any action was required. In this task the firing of individual ventral striatal neurons was related to reward size, delay, or both (ANOVA, significant main effects). In terms of population analysis, we asked if the coding of the reward size and the delay has an aspect in common across the ventral striatal neurons. Specifically, we explored if the neural responses were related enough so that they can all be treated as if there were related mappings between the experimental factors and neural firing. To this end, we considered all of the neurons as a single group in a generalized linear mixed-effects model (GLMM), with neurons and the number of trials as random factors, and linear and quadratic terms of reward size and delay as fixed factors. The neural responses were well predicted by the GLMM when both linear and quadratic terms were included for the fixed effects for both monkeys.

Poster

539. Songbird Communication: Genetic, Neuroendocrine, and Environmental Influences

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#Poster#: 539.01/CC1

Topic: F.04. Neuroethology

Support: NIMH Grant R01 MH080225

Title: Neurotensin mRNA expression in the medial preoptic nucleus and Area X positively correlates with sexually-motivated song in male European starlings

Authors: *D. P. MERULLO, M. A. CORDES, M. S. DEVRIES, S. A. STEVENSON, L. V. RITERS; Zoology, Univ. of Wisconsin-Madison, Madison, WI

Abstract: Vocalizations coordinate social interactions in many species and often are important for behaviors such as mate attraction or territorial defense. Although the neural circuitry underlying vocal communication is well-known for some species, such as songbirds, the motivational processes that regulate vocal signals are not as clearly understood. Neurotensin (NT) is a neuropeptide implicated in motivation that can modulate the activity of dopaminergic neurons. Dopaminergic projections from the ventral tegmental area (VTA) are key to mediating highly motivated, goal-directed behaviors, including sexually-motivated birdsong. However, the role of NT in modifying vocal communication or other social behaviors has not been well-studied. Here in European starlings (Sturnus vulgaris) we analyzed relationships between sexually-motivated song and NT and NT1 receptor (NT1R) expression in VTA. Additionally, we examined NT and NT1R expression in three regions that receive dopaminergic projections from VTA and are involved in courtship song: the medial preoptic nucleus (POM), Area X, and HVC. Relationships between NT and NT1R expression and non-vocal courtship and agonistic behaviors were also examined. NT expression in POM and Area X positively related to sexually-motivated song production. NT expression in POM also positively correlated with non-vocal courtship behavior and agonistic behavior. These results are the first to implicate NT in the POM and Area X in birdsong, and further highlight NT as a potential neuromodulator for the control of vocal communication and other social behaviors.

539. Songbird Communication: Genetic, Neuroendocrine, and Environmental Influences

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 539.02/CC2

Topic: F.04. Neuroethology

Support: NIMH Grant R01 MH080225

Title: Independent contributions of testosterone and a nesting site to sexually-motivated behaviors and gene transcription in male European starlings

Authors: *J. A. SPOOL*, S. A. STEVENSON, C. S. ANGYAL, L. V. RITERS; Zoology, Univ. of Wisconsin Madison, Madison, WI

Abstract: In many vertebrates, acquiring a limited resource alters the production of sexually- and agonistically-motivated behaviors. For example, male European starlings that acquire a nesting site go from ignoring females and tolerating other males to vigorously courting females and defending territory from other males. Seasonal elevations in testosterone (T) are necessary but not sufficient to induce these behavioral changes. Males must also acquire a nesting site before they will display these behaviors. This suggests that obtaining a nesting site and a rise in T both contribute to shifting a starling’s motivational state. T or its metabolite estradiol (E2) are proposed to modulate sexually-motivated behaviors in part by altering activity in motivation circuits in the brain, such as dopaminergic systems, yet this has not been well studied. The medial preoptic area (POM) plays a role in sexual motivation, and androgen receptor (AR) immunolabeling in this region is greater in nest box owning birds with elevated T than in either males with high T or a nest box alone. If T and a nesting site are sufficient together but not separately to induce this shift in motivational state, then male starlings with both T and a nesting site, but not either alone, will alter gene transcription in the POM to support behavioral changes. To provide insight into this hypothesis, we examined the individual contributions of T and a nesting site to changes in male behavior and the transcription of genes important for dopamine and steroid hormone activity in the POM and other brain regions. Castrated male starlings were given subcutaneous empty or T-filled implants and introduced to an aviary with or without a nesting site for 10 days. Behavioral responses to the introduction of a female and nesting material were observed for 3 days, and neural tissue was subsequently collected. Quantitative real-time PCR (qPCR) was used to quantify relative changes in the mRNA expression of D1 and D2 dopamine receptors, AR, estrogen receptor alpha, and aromatase.

Disclosures: J.A. Spool: None. S.A. Stevenson: None. C.S. Angyal: None. L.V. Ritters: None.
Poster

539. Songbird Communication: Genetic, Neuroendocrine, and Environmental Influences

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 539.03/CC3

Topic: F.04. Neuroethology

Support: NIH Grant R01MH080225

Title: Endocannabinoid CB1 receptor expression in social and vocal control brain regions correlates with status-appropriate agonistic- and sexually-motivated behavior in male European starlings

Authors: *L. V. RITERS, M. S. DEVRIES, M. A. CORDES, J. D. RODRIGUEZ, S. A. STEVENSON;
Dept. of Zoology, Univ. of Wisconsin, Madison, WI

Abstract: Successful social interactions require individuals to adjust behavior to match emerging social or environmental circumstances, yet the neural mechanisms underlying status-appropriate behavior are not well characterized. European starlings provide a robust system for study of this topic. Prior to acquisition of a nesting territory, male starlings appear to ignore females and to avoid other males. In contrast, once a male acquires a nesting site he socially dominates other males, defends the nesting site, and sings high rates of courtship song. Past studies show that status-appropriate changes in behavior involve dopamine and opioids and are accompanied by altered activity in brain regions involved in social behavior and vocal production. Endocannabinoids richly innervate many of these regions, modulate dopaminergic and opioid systems, and are implicated in both agonistic and vocal behaviors; yet, the role they play in adjusting social behavior to match social status has not been explored. Here we observed flocks of male starlings in outdoor aviaries during the breeding season. Ten males acquired nesting sites and 10 males did not. Brains were collected and quantitative real time PCR was used to measure expression of the endocannabinoid CB1 receptor in social (lateral septum [LS], ventral tegmental area [VTA], and the medial preoptic nucleus [POM]) and vocal control regions (Area X and RA). Males with nesting sites sang to females and displaced other males more than males without nesting sites. They also had higher levels of CB1 receptor expression in both LS and RA. CB1 expression in VTA correlated positively with song rate. CB1 expression in LS correlated positively with territorial and agonistic behaviors. Finally, feeding behavior correlated negatively with CB1 expression in VTA and Area X but positively with expression in RA. Results are consistent with the possibility that CB1 expression in LS may gate status-appropriate agonistic/territorial behavior; whereas CB1 in VTA may gate sexually-motivated vocal
production. Associations between CB1 expression in RA and Area X are consistent with past studies showing food restriction to alter CB1 activity in auditory regions. Overall, results support prior work suggesting that endocannabinoid signaling functions as a behavioral switch, adjusting behavior so that it is appropriate given emerging environmental or social factors.


Poster

539. Songbird Communication: Genetic, Neuroendocrine, and Environmental Influences

Location: Hall A
Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM
Program/#/Poster#: 539.04/CC4
Topic: F.04. Neuroethology
Support: NSF IOS1354906
Title: Neuroestrogen modulation of auditory processing across development

Authors: *D. M. VAHABA¹, L. REMAGE-HEALEY²;
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Abstract: Brain-derived steroid hormones can act as neuromodulators of sensory encoding. Therefore, neurosteroids may play a significant role in facilitating the formation of auditory representations during development. In particular, 17β-estradiol (E2; a centrally synthesized neuroestrogen) enhances auditory encoding through increased neuronal firing in adult songbirds. Neuroestrogens may also participate in consolidating auditory memories required for vocal learning during the critical period of language acquisition in human infants, and song acquisition in birds. At present, it is unclear how E2 impacts auditory-evoked neuronal activity during early development. Here, we collected unit recordings in the caudomedial nidopallium (NCM) of juvenile and young adult male zebra finches (Taeniopygia guttata). NCM is a higher-order sensory area functionally homologous to secondary auditory cortex in mammals. Moreover, NCM is a requisite brain region for auditory memory consolidation, contains a significant population of aromatase-positive neurons, expresses nuclear and membrane-bound estrogen receptors, and shows dynamic E2 fluctuation in adult and developing zebra finches when presented conspecific song. Anesthetized subjects were presented with conspecific auditory playbacks during simultaneous retrodialysis of E2 and extracellular recordings in NCM. The neuromodulation of auditory-evoked neuronal activity was analyzed by subjects’ age/phase of
their song development. Preliminary findings indicate that the E2 modulation of auditory-evoked neuronal activity is age-dependent, divergent from prior findings on adult neuroendocrine function. This ongoing study extends our understanding of estrogen-dependent neuromodulation of auditory processing across development. This work was supported by NSF IOS1354906.

Disclosures:  D.M. Vahaba: None.  L. Remage-Healey: None.

Poster

539. Songbird Communication: Genetic, Neuroendocrine, and Environmental Influences

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 539.05/CC5

Topic: F.04. Neuroethology

Support: NSF IOS 0917918

Title: Neural representation of a shared behavior in two individuals

Authors: *M. J. COLEMAN*¹, A. ROESER², F. DUQUE³, E. S. FORTUNE²; ¹Keck Sci. Dept., The Claremont Colleges, Claremont, CA; ²Dept. of Biol. Sci., New Jersey Inst. of Technol., Newark, NJ; ³Univ. San Francisco de Quito, Quito, Ecuador

Abstract: Male and female plain-tailed wrens (Pheugopedius euophrys) sing duet songs in which participants rapidly alternate syllable production. The duet is so precisely timed that it sounds like one bird is producing it. The ongoing coordination of timing between individuals during duets is mediated in part via auditory feedback between the participating birds. Further, although the pattern of syllable production appears to be stable, each bird produces an unexpectedly large number of syllable variants. In other words, behavior evidence suggests that the songs of these birds are more like jazz riffs rather than highly stereotyped song performances. We want to understand the neural mechanisms for the temporal coordination these variable duet performances. We captured duetting pairs of wrens, recorded their duet singing over a period of one to two days, and then made acute (urethane anesthesia) extracellular recordings of neurons in HVC of both individuals. Auditory stimuli included duet variants, conspecific songs, and experimentally altered song stimuli. Previous work showed that HVC neurons respond more strongly to duet stimuli than to the individual performances of each bird. Here we want to examine the temporal structure of HVC activity to better understand how sensory and sensorimotor information is encoded and processed. Specifically, we predict that the timing of responses in each bird should map onto different time points in the duet. This timing of activity might correlate to time points used in the coordination of duet singing, or perhaps to the motor
output of each bird, or both. We recorded from three pairs of male and female wrens. Our initial analyses suggest a surprising result. Rather than the activity alternating in time between the two birds, as is seen in the behavior, the activity appears to be synchronized across birds. This result suggests that HVC activity is not a traditional form of direct premotor activity, but is used for the coordination of the timing of the singing performance. If the role of HVC, generally speaking, is the temporal coordination of song performances, then HVC may be the main site for the coordination of duetting.

Disclosures: M.J. Coleman: None. A. Roeser: None. F. Duque: None. E.S. Fortune: None.

Poster
539. Songbird Communication: Genetic, Neuroendocrine, and Environmental Influences

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 539.06/CC6

Topic: F.04. Neuroethology

Support: Hercules Foundation AUHA0012

Interuniversity Attraction Poles (IAP): ‘PLASTOCINE’: P7/17
Research Association - Flanders (FWO): PhD fellowship to LVR
Research Association - Flanders (FWO): Post-doc fellowship to GDG
Natural Sciences and Engineering Research Council, Canada

Title: Neural substrates of courtship song perception in female zebra finches: a role for the avian ‘prefrontal cortex’

Authors: *L. VAN RUIJSSEVELT*¹, Y. CHEN², G. DE GROOF¹, S. C. WOOLLEY², A. VAN DER LINDEN¹; ¹Bio-Imaging Lab. / Univ. of Antwerp, Antwerpen (wilrijk), Belgium; ²Biol., McGill Univ., Montreal, QC, Canada

Abstract: Like humans, zebra finches (ZF) adjust their vocal performance depending on social context. Adult male ZFs subtly alter the characteristics of their song when courting females compared to when singing alone. In particular, female-directed song (‘DIR’) is faster and has syllables with significantly less spectral variability than ‘undirected’ song (‘UDIR’) [1]. Previous data indicate that females prefer DIR to UDIR and that this behavioral preference is correlated
with greater EGR1 expression in a higher-level auditory area the caudomedial mesopallium (CMM) [1]. To investigate the neural representation of this social perception at the level of the whole brain, we used blood oxygenation level dependent (BOLD) functional magnetic resonance imaging (fMRI) in 17 adult isoflurane-anesthetized female ZFs. We assessed BOLD responses to DIR and UDIR stimuli and to manipulated versions of these 2 types of song (‘mDIR’, ‘mUDIR’) to study the role of specific acoustic features. mUDIR stimuli contained the DIR temporal pattern but were composed of UDIR syllables while mDIR songs contained DIR spectral and temporal characteristics but controlled for the manipulation. We found higher, lateralized neural activation for DIR vs UDIR in three distinct brain regions: the CMM, HVC (proper name) and the caudolateral nidopallium (NCL; Figure 1). In addition, the results indicate that this selective higher response to DIR in the CMM is preserved for mDIR but not mUDIR vs UDIR. In contrast, HVC and the NCL appeared to be only selectively activated by the unmanipulated DIR songs. Together these data indicate a potential gradual specialization of the network for specific acoustic features. Moreover, through visualization of responses of the entire brain to socially relevant stimuli, fMRI highlights the potential role of regions beyond the auditory system, including the NCL, which is the avian analogue of the prefrontal cortex [2], in the neural processing of acoustic social cues. **References:** [1] Woolley and Doupe, 2008, PLoS Biol; [2] Güntürken, 2005, Curr Opin Neurobiol

**Disclosures:** L. Van Ruijssevelt: None. Y. Chen: None. G. De Groof: None. S.C. Woolley: None. A. Van der Linden: None.

**Poster**

539. Songbird Communication: Genetic, Neuroendocrine, and Environmental Influences

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 539.07/CC7

**Topic:** F.04. Neuroethology
Support: NSERC Discovery Grant 402186-11

Title: Pair bond quality influences song preferences and EGR1 expression in a female songbird

Authors: H. E. SCHUBLOOM¹, *S. C. WOOLLEY²;
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Abstract: Pair bonding has been characterized in a handful animal species, and considerable progress has been made in understanding the neurobiology of social interactions and affiliation that lead to and immediately follow pair bonding. However, given the complexity of pair bonding, we know substantially less about how the intricate relationships between individuals shape brain and behavior. For example, little is known about how the history of interactions between paired individuals influences their subsequent interactions, or their perception of and preference for their mate, especially in animal models. Here, we investigated how variation in social relationships in a pair bonding songbird, the zebra finch, relates to female song preferences and neural responses to song. We assessed variation in the types of interactions between individuals in a pair and found that the nature of those interactions was correlated with the degree of female preference for her mate’s song over the song of an unfamiliar male. In particular, female preferences were influenced most strongly by whether their mate performed more courtship or non-courtship song. In addition, we investigated how pair quality affected the neural expression of the immediate early gene EGR1 in response to playback of the mate’s courtship song. In songbirds, EGR1 expression has been associated with auditory memory as well as the perceived quality or salience of song, making, EGR1 expression a useful metric for investigating neural responses to socially relevant song stimuli. We found that variation in pair quality was correlated with the amount of EGR1 expression in females in response to their partner’s song in nuclei within the associative auditory cortex and the social behavior network. Our data highlight that the quality of social interactions within a pair influences female perception, behavioral preferences and neural activity in response to categorically similar stimuli, perhaps through changes in activity in auditory and social behavior neural networks.

Disclosures: H.E. Schubloom: None. S.C. Woolley: None.

Poster

539. Songbird Communication: Genetic, Neuroendocrine, and Environmental Influences

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 539.08/CC8

Topic: F.04. Neuroethology
Title: Male lays eggs: chromosomal and mate preference abnormalities in a chimeric zebra finch lineage

Authors: *M. JONES¹, R. A. CUMMINS¹, E. JENKINS¹, R. J. PEREZ², M. BARKER-KAMPS¹, H. WITTCHEN¹, C. GANN-VACULCIK¹, A. L. HRIBAR¹, L. B. DAY¹;
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Abstract: In birds, males have ZZ sex chromosomes and females have ZW sex chromosomes. In zebra finches, sexually dimorphic plumage patterns are determined by genes on the sex chromosomes. Steroid hormones are known to organize aspects of the reproductive system and nervous system while also activating growth of the neuromuscular male song system and singing. The exact contribution of genes on sex chromosomes, genes on autosomes, and circulating hormones on sexual differentiation in zebra finches is not completely understood. Birds that have mixed ZZ and ZW chromosomes in different tissues of their body can help us understand the role of sex chromosome genes in sexual differentiation. We discovered a bird in our aviary that has male plumage, lays eggs, has a apparently seemingly typical male partner, and produces viable offspring with this partner. Using PCR for two different sex-linked genes, we found the suspected chimera does not have a W-linked gene in the blood but a W-linked gene is present in its unfertilized eggs. We also found that in all female-plumage offspring, a W-linked gene is present, while in the male-plumage offspring a W-linked gene was not present. We are currently analyzing feather samples and other tissues for multiple W-linked genes in the chimera and its offspring. In addition to examining the linkage between genes and sexual differentiation, this unusual bird and its offspring allow us to explore mate choice for visible plumage characteristics versus cryptic abnormalities that may be present in chimeric offspring. Using a two-choice mate preference paradigm, we tested whether our chimeric bird, its partner, and their offspring were preferred as highly as control birds in our aviary, and if birds in the chimeric lineage preferred opposite sex-plumaged birds at frequencies comparable to typical zebra finches. We used the two-choice mate preference test counterbalanced across days for sides, and tracked the proportion of the total 30 minute time chooser birds spent with each choice bird after a 2 hour habituation period. Our data suggests the chimeric line has cryptic abnormalities that are not preferred by other birds in our aviary and that the chimeric line has abnormal biases for same sex birds.


Poster

539. Songbird Communication: Genetic, Neuroendocrine, and Environmental Influences

Location: Hall A
Title: Anatomical specificity of testosterone in the regulation of song and the associated neuroplasticity in canaries

Authors: *B. A. ALWARD¹, S. E. PARKER¹, J. BALTHAZART², G. F. BALL¹,³; ¹Psychological & Brain Sci., The Johns Hopkins Univ., Baltimore, MD; ²GIGA Neurosciences, Univ. of Liege, Liege, Belgium; ³Psychology, Univ. of Maryland, Col. Park, College Park, MD

Abstract: Steroid hormones can affect multiple features of complex social behaviors and the interconnected brain regions that subserve these behaviors. A fundamental question is how steroid hormones acting in distinct brain areas coordinate the activation of a complex behavior. In songbirds, a circuit called the song control system (SCS) regulates many different aspects of song behavior. In the posterior SCS, HVC (acronym is name) projects to the robust nucleus of the arcopallium (RA)—these two nuclei regulate the production of song. RA in turn projects through the hindbrain to regulate the muscles of the syrinx, the avian vocal organ. HVC also projects to Area X, an anterior striatal nucleus critical for learning song. HVC and RA are characterized by cells expressing a high density of androgen receptors. Moreover, T in the medial preoptic nucleus (POM) is critical for regulating the motivation to sing but not the acoustic structure of song. During the spring when T is high, birds sing more and with higher quality and the SCS almost doubles in volume compared to the winter months. Notably, HVC recruits more new neurons during these spring conditions. Also, singing activity can drive SCS neuroplasticity independently of T acting directly in the SCS. Hence, while it is clear that T regulates plasticity of song and the SCS, it is unclear where T acts to coordinate these changes.

We investigated these questions using canaries. We castrated males and let them acclimate in sound-attenuated chambers set on a photoperiod of 14L:10D to simulate long days associated with the breeding season for 1 week. Birds were either treated with T peripherally (PER-T) so it acted everywhere, T implanted in the POM and HVC (HVC-POM T), T only in the POM (POM-T), T only in HVC (HVC-T) or with no T contacting either HVC or POM (HVC-POM NO T). We recorded song for two weeks and then collected all brains. We confirmed implant sites and the volumes of HVC, RA, and Area X using Nissl stains. We also stained for doublecortin (DCX), a protein that marks new neurons. T in the POM increased song rate to PER-T levels. Birds with HVC-POM T produced higher quality songs compared to POM-T birds and no different from PER-T birds. Singing activity in itself increased the volume of the SCS but T acting only in HVC also drove such changes. T in HVC caused a recruitment of neurons to this nucleus but song may also play a role in causing these neurons to undergo differentiation. Thus steroid hormones act directly and indirectly to modulate behavioral output and the underlying
neural substrate. These results highlight the complex roles played by steroid hormones in the regulation of brain and behavior.

Disclosures: B.A. Alward: None. S.E. Parker: None. J. Balthazart: None. G.F. Ball: None.

Poster

539. Songbird Communication: Genetic, Neuroendocrine, and Environmental Influences

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 539.10/CC10

Topic: F.04. Neuroethology

Title: Acceleration of maturation by estradiol during adolescence in prepubescent male zebra finches

Authors: *W. E. GRISHAM1, N. ASKARINAM1, M. NELSON2, I. T. DAHLIG3, A. A. CARLSON4, D. SAXON4;
1Dept Psychol, 2Mathematics, UCLA, Los Angeles, CA; 3Dept. of Chem., Trinity Col., Hartford, CT; 4Dept. of Neurosci., Claremont McKenna Col., Claremont, CA

Abstract: The song system is sexually dimorphic: vocal control nuclei are up to six times larger in males than females (Nottebohm & Arnold, 1976) and most song regions have larger neurons and dendritic extensions (DeVoogd & Nottembohm, 1981). These sex differences in neural structure are manifested behaviorally males engage in singing, unlike females. Implanting estradiol (E2) in young females masculinized the song system up to post-hatching day 40 (Konishi & Akutagawa, 1988). Little work has yet to be done on examining the effects of estradiol in adolescent males. Our study examined the effects of administering estradiol on the sexual differentiation of the song system’s neural circuitry in prepubescent males. Ten subjects at 28-33 days of age were assigned to groups: treated males (n=4), implanted controls (n=4) or non-implanted controls (n=2). Treated males were implanted with 4-5 mm Silastic pellets packed with estradiol, which results in a slow, sustained release of hormone. On day 39-45 subjects were sacrificed; brains were fixed in formalin, frozen sectioned at 40 microns, and stained with thionin. Dependent variables included volume, cell density, cell counts, size and total number of neurons in IMAN, RA and HVC. Volume of Area X was also examined. Cell counts and sizes were examined under 400X. E2-treated males had a significantly greater number of neurons in HVC than controls; t(8) = 2.05, p = 0.037. This result was paralleled with a trend toward larger HVC volume found in E2-treated males compared to controls; t(8) = 1.80, p = 0.055. Anecdotally, the treated birds were brought into full song precociously. No other variables including volumes of Area X, RA, and IMAN, cell sizes in HVC, RA and IMAN, and cell counts
in RA and IMAN were significantly different between treated and control birds. Estradiol may have its impact on the song system via its ability to upregulate the expression of brain-derived neurotrophic factor (BDNF), which may in turn stimulate neuronal migration, proliferation and survival. BNDF mRNA expression in HVC was higher in estrogen-treated 20-25 day old males than their age-matched controls (Dittrich et al., 1999). Once the estrogen implant was removed, HVC’s BNDF expression dropped (Dittrich et al., 1999). Therefore, estradiol could have increased the number of HVC neurons, and consequently HVC volume via its upregulation of BDNF.


**Poster**

539. Songbird Communication: Genetic, Neuroendocrine, and Environmental Influences

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 539.11/CC11

**Topic:** F.04. Neuroethology

**Support:** NSHRF Development Grant 1528

NSERC Discovery Grant

**Title:** Coping with stress: does having a single parent affect offspring of typically biparental zebra finches (Taeniopygia guttata)?

**Authors:** *L. S. PHILLMORE, J. FISK, S. D. AITKEN, T. M. E. YOUSEF, T. S. PERROT; Psychology and Neurosci., Dalhousie Univ., Halifax, NS, Canada

**Abstract:** Stressful events during childhood, such as having a single parent, shape how a child's body and brain respond to stressors later in life. Previous work modeling the effects of developmental stress have primarily used a rodent model, however a disadvantage of using rats, for example, is that only mothers provide care for the young, meaning the potential contribution of fathers to offspring rearing, and the effects of single vs. biparental care cannot be examined. Avian species such as zebra finches typically provide biparental care, and therefore we can examine how removal of one parent (either the father or the mother) affects both the remaining parent and the offspring. There is some research on how single zebra finches attempt to compensate for the lack of a mate behaviourally, but research on single fathers is minimal. In this study we examined how being a single parent and having a single parent affected behaviour (e.g.
feeding, begging), physiology (corticosterone (CORT levels), and the brain (glucocorticoid receptor GCR levels). Offspring reared by single parents did not weight less than offspring reared by both parents, however, single males compensated differently than single mothers. Single parents did not have increased circulating CORT compared to parents with a partner, and CORT results for offspring were mixed. These preliminary results may indicate that offspring of single zebra finches respond with resilience, rather than compromise.


Poster

539. Songbird Communication: Genetic, Neuroendocrine, and Environmental Influences

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 539.12/CC12

Topic: F.04. Neuroethology

Support: NSERC 402417-2011

FQRNT 144721

Title: Predicting plasticity: Context-dependent changes to vocal performance predict age-dependent changes to adult birdsong

Authors: L. S. JAMES1, *J. T. SAKATA2;
1Biol., 1McGill Univ., Montreal, QC, Canada

Abstract: The performance of ethologically important behaviors can change substantially over an individual’s life. Understanding the factors that predict and guide such behavioral change can lend insight into mechanisms of motor plasticity and individual differences in behavior. The performance of adult birdsong changes with age; the songs that young adult Bengalese finches produce in isolation (undirected or UD songs) become faster and more stereotyped over time. The extent to which these features change varies among individuals, and little is known about how to predict such variation in plasticity. Social context affects song in ways that resemble age-dependent changes: the songs that young adult finches direct to females (female-directed or FD songs) are faster and more stereotyped than their UD songs. As such, we investigated the degree to which variation in the direction and magnitude of context-dependent changes predicted variation in age-dependent changes to adult Bengalese finch song. Using a repeated-measures design, we found that variation in context-dependent changes to inter-syllable gap durations,
variability of syllable sequencing, and probabilities of individual sequence transitions significantly predicted variation in age-dependent changes to such features. On the other hand, variation in context-dependent changes to syllable structure (fundamental frequency) provided less predictive insight into variation in age-dependent plasticity. The temporal structure of a bird’s UD song converged over time onto the structure of the FD song that the bird produced as a young adult. One model that could explain this convergence is that the FD song of young adults represents a stable target for age-dependent changes to song. This model predicts that FD song remains unchanged over time and that, since the UD song of older adults have reached this target, older birds should no longer exhibit context-dependent changes to song. An alternative explanation is that age- and context-dependent changes represent changes to vocal performance. According to this model, the performance of FD song could also change over time, and social context could continue to affect vocal performance in older adults. Consistent with the performance but not target model, we found that the FD songs produced by birds as older adults were faster and more stereotyped in sequencing than both the FD songs these birds produced as young adults and the UD songs these birds produced as older adults. Overall, these data suggest that age-dependent changes reflect changes in vocal performance and that mechanisms regulating vocal performance could shape vocal motor plasticity.


Poster

539. Songbird Communication: Genetic, Neuroendocrine, and Environmental Influences

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 539.13/CC13

Topic: F.04. Neuroethology

Support: NIH Grant R24-GM092842

NIH Grant R24-GM089700

Title: Differential and developmental expression of genes with potential involvement in the specificity, maintenance, and modulation of long-distance projections in the oscine song system

Authors: *C. V. MELLO, C. R. OLSON, M. WIRTHLIN, P. V. LOVELL;
Dept. of Behavioral Neurosci., Oregon Hlth. Sci. Univ., Portland, OR

Abstract: The oscine song control system consists of discrete interconnected forebrain nuclei that play distinct roles in the acquisition and production of learned vocalizations. While these
nuclei have been extensively studied in terms of cell composition, connections, and electrophysiological properties; much less is known about the genetic factors that regulate the formation and maintenance of the projections that interconnect them. Molecular screenings (e.g. with microarrays) and in situ hybridization studies conducted while building our zebra finch molecular atlas of brain gene expression (ZEBrA; http://www.zebrafinchatlas.org/) have revealed that several genes with known roles in the establishment and specificity of long-distance axonal connections are differentially expressed in song nuclei of adult male zebra finches. These findings suggest that markers such as SEMA3E, SEMA6A, PLXNC1, NRP1, UNC5A, and RELN, which are known to participate in specific ligand/receptor interactions based on data from other organisms, may play a role in the formation and/or maintenance of song system projections. To gain further insights into the role these genes might play, we have used in situ hybridization with non-radioactive riboprobes to study the expression of these molecular markers along with their presumed ligand/receptor partners (i.e. SEMA3E/PLXND1 and NRP1, SEMA7A/PLXNC1, SEMA6A/PLXNA4, LRRCD4C/NTNG1 and G2, RELN/VLDLR and LRP8) in adults and in juvenile male zebra finches during the early phases of the formation of the song system connections. Our results provide evidence for the differential and developmental regulation of these markers in song nuclei and/or their projection targets, further supporting a possible role in the formation or maintenance of specific projections, including HVC to X, HVC to RA, LMAN to RA, and RA to midbrain/brainstem targets (e.g. DM, nXIIts). These studies lay important groundwork for future studies that will use gene manipulation strategies to investigate the functional role that these genes play in establishing connectivity in the song system.


Poster

539. Songbird Communication: Genetic, Neuroendocrine, and Environmental Influences

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 539.14/CC14

Topic: F.04. Neuroethology

Support: NIH Grant R24-GM092842

Title: ZEBrA Redux: An improved digital atlas for exploring brain gene expression in the adult male Zebra Finch (www.zebrafinchatlas.org)

Authors: *P. V. LOVELL¹, M. WIRTHLIN¹, C. V. MELLO²;
¹Dept. of Behavioral Neurosci., ²Behavioral Neurosci., Oregon Hlth. & Sci. Univ., Portland, OR
Abstract: ZEBrA (Zebra Finch Expression Brain Atlas; www.zebrafinchatlas.org) is a publicly accessible online collection of high-resolution (0.46 µm/pixel) digital images representing the expression patterns for a large set of transcripts in brain of the adult male zebra finches (T. guttata), a representative songbirds species. ZEBrA represents the most comprehensive resource available for investigating the brain distribution of genes involved in the physiology, development, and maintenance of functional circuits in the brain of songbirds. Each gene has been selected based on its relevance for understanding the physiology of the song system (e.g. neurotransmitter receptors, ion channels, signaling systems), or its importance for clarifying issues of vertebrate brain evolution and homology. Among its major features, ZEBrA contains: (1) The in situ database – the actual collection of high-resolution in situ hybridization images presented along with annotated drawings derived from Karten/Mitra Histological Atlas. ZEBrA currently houses more than 2,000 images (>100 GB) corresponding to several hundred genes expressed in the zebra finch brain, including the major nuclei that comprise the song system; (2) A Gene Family Search Page – A feature that facilitates searches for genes based on their membership in specific gene families; (3) A Histological Atlas Browser – A set of 18 annotated drawings prepared in registration with Nissl- and Myelin-stained images of sagittal brain sections derived from the Karten/Mitra atlas; and (4) A Neuroanatomical Marker Search Page - A search engine that allows users to retrieve a list of genes that are markers of a given structure, or of multiple structures. The main update reported here is the addition of 200 patterns, including a range of molecular markers that are high relevance for understanding brain evolution, and a suite of genes that are physiological important to the biology of songbirds. Our on-going mission is to continue to release additional batches of images until we have covered ~1,500-2,000 genes.


Poster

539. Songbird Communication: Genetic, Neuroendocrine, and Environmental Influences

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 539.15/CC15

Topic: F.04. Neuroethology

Support: NIH Grant R24-GM092842

Title: Promoter motif analyses reveal unique transcriptional regulatory networks in distinct cell types within the oscine song system

Authors: *M. WIRTHLIN, P. V. LOVELL, C. R. OLSON, J. CARLETON, C. V. MELLO; Behavioral Neurosci., Oregon Hlth. & Sci. Univ., Portland, OR
Abstract: The oscine song control system is comprised of a unique collection of cell types that possess anatomical and physiological properties that are specialized for the acquisition and production of learned vocalizations. These properties are, in turn, determined by the distinct transcriptional networks that co-regulate the expression of genes in each cell type. Identifying these networks is a critical first step towards identifying the underlying genomic features (e.g. promoter motifs) that determine the anatomical and physiological properties of these circuits. Unfortunately, disentangling and characterizing the gene expression profiles of unique cell types within heterogeneous structures has proven technically challenging. To overcome this challenge, we have recently examined the expression of genes in the premotor nucleus HVC of the zebra finch. HVC, required for the production of learned song, is comprised of several distinct cell types, which have been well characterized in terms of their connectivity, electrophysiological properties, and dynamics of adult replacement; properties that are likely to be supported by distinct gene networks. To identify these networks we have developed a novel suite of in silico promoter motif analysis algorithms that identify key regulatory modules within co-expressed gene networks. These analyses are guided and validated by molecular techniques (e.g. in situ hybridization). Briefly, we started with a 'seed' set of known molecular markers of HVC projection neurons previously identified by co-localizing expressed gene transcripts with retrogradely labeled cells. By identifying DNA binding motifs common to the promoters of these seed sets, we were then able to identify, and validate by *in situ* hybridization, an additional cohort genes that were predicted to be co-expressed in each cell type. This process was continued iteratively in order to identify regulatory elements motifs predictive of cell type expression, and to build gene expression networks that characterize these cell types. The identified gene networks include previously known and unknown cell type markers, provide candidate molecular substrates for the known properties of these song system cell types, and reveal previously unknown functional pathways that may be critical for various aspects of vocal learning. Our study also provides proof-of-principle for a novel methodology for identifying gene regulatory networks within cell types within complex neural structures.


Poster

539. Songbird Communication: Genetic, Neuroendocrine, and Environmental Influences

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 539.16/CC16

Topic: F.04. Neuroethology
Title: The NAP snippet of activity-dependent neuroprotective protein (ADNP) is highly conserved in migrating and monogamous non-song birds

Authors: *I. GOZES*, G. HACOHEN KLEIMAN1,2, A. YEHESKEL3, A. BARNEA2;
1 Sackler Sch. Med/Tel Aviv Univ., Tel Aviv, Israel; 2 Open Univ., Raanana, Israel; 3 Life Sciences/Tel Aviv Univ., Tel Aviv, Israel

Abstract: Activity-dependent neuroprotective protein (ADNP), discovered and extensively studied at the laboratory of Prof. Illana Gozes, is crucial for brain development and function in mice and men. Despite great evolutionary distance, ADNP of the songbird zebra finch was found to be highly homologous to human ADNP. As we recently reported, ADNP mRNA presents a sexually dichotomous expression in human and mouse with a significantly higher expression in the male hippocampus [1]. In our latest paper, we presented dichotomous and age-dependent ADNP mRNA expression in the zebra finch brain [2]. In young birds, ADNP was mainly expressed in the cerebrum with higher concentrations in males compared to females. With aging, ADNP levels dramatically decreased in the cerebrum of both males (3-fold) and females (2-fold). The high ADNP conservation suggests an important function throughout evolution and a significant function in neurodevelopment in birds. Here, bioinformatics comparative analyses of the protective ADNP snippet, NAPVSIPQ (NAP), sequence among 48 bird species, was performed. We compared singing behavior, social behavior, breeding strategy (monogamous or polygamous), migration/sedentary and male parental care. Results revealed a highly conserved NAP sequence in non-passerine species compared to passerine ones, especially in migratory and monogamous birds. This conservation in the NAP sequence in migrating and monogamous birds points out the importance of studying ADNP in birds exhibiting both migrating and social behaviors [2].


Disclosures: I. Gozes: None. G. Hacohen Kleiman: None. A. Yehekel: None. A. Barnea: None.
Title: Autonomously tunable interfaces for intracellular recordings

Authors: *S. SAMPATH KUMAR, J. MUTHUSWAMY; Arizona State Univ., Arizona State Univ., Tempe, AZ

Abstract: Current technologies used to record intracellular potentials have three major drawbacks: 1) they are large, bulky and require cumbersome positioning systems for steering their tips to neurons in specific circuits 2) their use requires extraordinary skill and tedious operations 3) the sheer size and weight of the system allows recording only from one neuron or a few neurons belonging to different neural circuits at a time. Here, we will present a novel microscale robotic intracellular recording system that has 3 main features: 1) electrothermal microactuators that allow microscale navigation in brain and precise positioning of electrode inside neuron; 2) polysilicon microelectrodes integrated with glass micropipettes to penetrate neurons and record intracellular potentials; 3) closed loop control algorithm to enable autonomous isolation and impalement of neurons. In this study, we demonstrate the ability of this technology to autonomously isolate, penetrate and record from single neurons in abdominal ganglion of *Aplysia* californica. The performance of the system was assessed in n= 50 attempts of single neuron penetrations. The results of yield and quality of neuronal recordings after the penetrations were carefully quantitated. Resting membrane potentials of 40 mV and action potentials ranging from 60-70 mV were consistently recorded in every attempt. Experiments to test the above technology in rodent brain slices are ongoing. The proposed system offers the unique advantages of significant reduction in size and weight, ability to seek neurons autonomously and readily scalable approach to realize multi-channel intracellular recording system.

Disclosures: S. Sampath Kumar: None. J. Muthuswamy: None.
Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 540.02/CC18

Topic: G.04. Physiological Methods

Title: Using multi-electrode array technology to evaluate in vitro neuronal firing parameters and network complexity

Authors: *R. Kesilman (Korn), S. Parmentier-Batteur, J. J. Renger, M. J. Marino; Early Discovery Neurosci., Merck & Co., Inc., West Point, PA

Abstract: Multielectrode Arrays (MEAs) enable simultaneous extracellular recording from multiple neurons in culture to evaluate in vitro firing parameters and network complexity. This technology has the potential to inform on cellular phenotypes expressed at the network level which may be more subtle when studied by single cell or biochemical methods. Changes in these in vitro parameters may provide an assay of cellular phenotype underlying complex psychiatric disorders and could therefore enable the identification and validation of novel molecular targets. The goal of the present study was to utilize the MEA platform to investigate NMDA receptor blockade-induced changes in in vitro firing parameters and network complexity in rat hippocampal neurons. Neurons were cultured on electrode arrays in the presence of the NMDA receptor antagonist D-APV (25 µM) beginning on day 5 in culture, and cell activity was recorded from day 5 until day 21 in culture. Firing of neurons in culture was evaluated using traditional time series analytic methods to evaluate firing frequency, inter-spike interval (ISI), and the coefficient of variation of the ISI (ISI CV). Time series analysis suggested that treatment with D-APV had no significant effect on the number of active neurons in the cultures, firing frequency, or ISI. However it was noted that the variability in these statistics was higher in untreated cultures. Consistent with this observation, the ISI CV was significantly decreased by D-APV treatment at 12-16 DIC (p<0.05 2 Way ANOVA, Sidak post hoc). Theoretical network structure was assessed through correlation of firing pattern and graph theory analysis. This analysis suggested that the networks formed under control conditions were highly dynamic and would increase and decrease in complexity during the 21 DIC. In contrast, networks formed in the presence of D-APV tended to monotonically increase in complexity. This observation was supported by a significant effect of D-APV on the network global clustering coefficient (2 Way ANOVA, p<0.05) and a large decrease in the variability of this statistic from DIC 12 on. Taken together, these results suggest that D-APV treatment in this in vitro system leads to a decrease in network plasticity resulting in the premature locking of network structure. Current efforts are directed at the use of high content imaging to determine if these network measures correlate with markers of synapse formation and plasticity.

Renger: A. Employment/Salary (full or part-time); Merck & Co., Inc. M.J. Marino: A. Employment/Salary (full or part-time); Merck & Co., Inc.

Poster

540. Electrodes Arrays II

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 540.03/CC19

Topic: G.04. Physiological Methods

Support: BSAC

Title: Silicon carbide-based electrocorticography arrays for chronic implantation

Authors: *C. DIAZ-BOTIA*1,2, L. E. LUNA1, M. CHAMANZAR3, C. CARRARO3, R. MABOUDIAN4, P. N. SABES2, M. M. MAHARBIZ2;
1Univ. of California, San Francisco, San Francisco, CA; 2UCB-UCSF Grad. Group in Bioengineering, 3Chem. and Biomolecular Engin., 4EECS, Univ. of California Berkeley, Berkeley, CA; 5Physiol., Univ. of California San Francisco, San Francisco, CA

Abstract: Several technologies have been developed for interfacing with the brain such as microwires, electrode arrays, and electrocorticography (ECoG) arrays. While each of them has strengths and weaknesses, they all share a common disadvantage of limited device longevity due to a variety of failure modes; these include scar tissue formation and material failure, among others. A particularly pronounced problem is the failure of the insulating material at the insulator-conductor interfaces (e.g. recording sites and insulated conducting traces). Damage to these vital interfaces compromises device performance by altering the impedance of recording sites, or more deleterious, results in total device failure due to shorting between traces or between a trace and physiological fluid. To address these material issues, we have focused on the fabrication of silicon carbide (SiC) electrode arrays. As a surface coating, polycrystalline SiC has been shown to promote negligible immune glial response compared to bare silicon when implanted in the mouse brain. Additionally, due to its mechanical and chemical stability, SiC serves as a stable platform and excellent diffusion barrier to molecules present in the physiological fluid. Moreover, and of particular interest to the neuroengineering community, the ability to deposit either insulating or conducting SiC films further enables SiC as a platform material for robust devices. Leveraging these unique properties, we have developed a fabrication process that integrates conducting and insulating SiC into 64-channel ECoG arrays. Recording sites 40 um in diameter are made of n-doped SiC while the insulating layers are either amorphous SiC or undoped polycrystalline SiC. To allow for low impedance interconnects, a metal stack of
titanium/gold/titanium or a single layer of molybdenum is completely embedded in between layers of SiC. The result is an ECoG array that, to the physiological fluid, appears simply as a single SiC sheet wherein boundaries between conducting and insulating layers are seamless. The inner metal layer is well protected by SiC and therefore cannot be reached by molecules present in the physiological fluid. We believe this basic platform can be extended to a variety of electrophysiological devices, including penetrating probes of various geometries, and help mitigate the failure modes of the present technologies.


Poster

540. Electrodes Arrays II

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 540.04/CC20

Topic: G.04. Physiological Methods

Support: Craig H. Neilsen Foundation Grant 314980

Title: Dorsal root ganglia neural recordings with a novel non-penetrating thin-film microelectrode array

Authors: *Z. J. SPERRY, J. P. SEYMOUR, F. WU, S. E. ROSS, K. KIM, J. T. BENTLEY, E. YOON, T. M. BRUNS; Univ. of Michigan, Ann Arbor, MI

Abstract: A neural interface with dorsal root ganglia (DRG) can provide a rich source of sensory neural activity from peripheral limbs and organs. There are presently no devices designed specifically for interfacing with DRG, so penetrating electrode arrays designed for the brain are typically used. Cortical arrays are inefficient DRG interfaces as they are not designed for the small, curved features of spinal roots, and their penetrating shanks can cause tissue trauma and immune responses. A recent study demonstrated single-unit neural activity from the DRG surface, taking advantage of superficial cell bodies, though the electrodes were not suitable for long-term recordings and required downward force to yield signals. To address these shortcomings, a new thin-film electrode array was designed to provide a close fit to the surface of the DRG. The 64-channel electrode array was microfabricated on an ultrathin (3.6 µm) polyimide substrate to provide flexibility to conform to the natural curvature of the spinal roots (~1 mm radius). The iridium electrode sites (1130 µm2) have varying pitch (25-300 µm), with
impedances of $160 \pm 24 \text{k}\Omega$ at 1 kHz. Functionality of the array was tested \textit{in vivo} in an anesthetized feline model after a lumbosacral laminectomy. When the array was placed on lumbar DRG, surface tension yielded a contoured fit to the tissue without the need for additional downward force. Thresholded neural activity was manually sorted offline using commercial spike sorting software. Single-unit activity associated with a tactile stimulus was observed on 18 unique channels, and multi-unit activity associated with a tactile stimulus was observed on 27 unique channels. The mean signal to noise ratio for these signals, calculated as peak to peak amplitude over three times the noise standard deviation, was 1.85 with a standard deviation of 0.57. These results demonstrate the ability of this novel surface array to record high-fidelity neural signals at the DRG. Future work will include optimization of the electrode and studies with chronic placement.


Poster

540. Electrodes Arrays II

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 540.05/CC21

Topic: G.04. Physiological Methods

Support: National Science Foundation Graduate Research Fellowship

Ford Foundation Predoctoral Fellowship

Rackham Merit Fellowship

Title: Data-driven model comparing the effect of the glial scar and electrochemical interface on chronic neural recordings in non-human primates

Authors: *K. A. MALAGA$^1$, K. E. SCHROEDER$^1$, P. R. PATEL$^1$, Z. T. IRWIN$^1$, D. E. THOMPSON$^1$, J. N. BENTLEY$^2$, C. A. CHESTEK$^1$, P. G. PATIL$^{1,2}$;

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Abstract: The ability to reliably record single-unit activity with intracortical microelectrode arrays is hindered by low signal-to-noise ratio. This can stem from a variety of biological, material, or mechanical failure modes, with the chronic foreign body reaction being one of the more studied failure mechanisms. In this study, we characterize electrode stability over twelve
weeks of electrophysiology recordings from four chronically-implanted Utah arrays in two rhesus macaques and quantify the effect of the glial scar and electrochemical interface on recording quality using a data-driven model. A finite-element model of a single Utah array microelectrode implanted in neural tissue was coupled with a compartmental model of a neuron to analyze the effect of encapsulation thickness, encapsulation resistivity, and interface resistivity on electrode impedance and waveform amplitude. The coupled FEM-neuron model was then reconciled with the neural data. Histology was obtained from one subject to assess the extent of gliosis. Mean impedance increased at a rate of $115.8 \pm 239.3$ kΩ/week during the first three weeks post-implantation. During this time, mean amplitude increased at a rate of $23.1 \pm 48.9$ µV/week. This initial ramp in impedance and amplitude was observed across all four arrays, and is nominally consistent with biofouling and reduction of edema, respectively, in the mathematical model. Beyond the third week, mean impedance ($-0.7 \pm 47.4$ kΩ/week) and amplitude ($-1.1 \pm 11.7$ µV/week) stabilized. Histology confirmed that relatively thin scars ($16 \pm 10$ µm) formed around the implanted electrodes. In the model, macroscopic scarring alone was not enough to account for the large impedance changes observed experimentally. However, the addition of a thin interface layer around the recording site was able to match the data. While interface resistivity had a large effect on impedance, the model did not predict it to have a significant effect on amplitude. Overall, this study suggests that the glial scar does not cause an electrical problem. Specifically, macroscopic scarring does not appear to: (1) be the principal contributor to increased electrode impedance over time, and (2) impact waveform amplitude, unless it is thick enough to displace nearby neurons. This, in turn, suggests that if the recording site is able to coexist with the first few microns of biofouling, then neural signals can still be obtained reliably. Therefore, microelectrodes may be improved by focusing on the interface between the recording site and scar tissue, and its accompanying impedance increase.


Poster
540. Electrodes Arrays II

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Topic: G.04. Physiological Methods

Title: An increased throughput platform for acute slice electrophysiology with microelectrode arrays

Deleted: in vitro
Authors: *M. S. TRUJILLO*¹, S. YASUOKA²;
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Abstract: *In vitro* multielectrode arrays (MEA) offer many unique advantages for probing the electrophysiological properties of excitable tissue. These advantages can be applied to investigating neuronal models of learning and memory, development, aging, disease, and much more. While several high-throughput MEA platforms have been developed in recent years to address electrophysiological properties in cultured cell applications, there have been limited platforms designed to study such attributes in acute or cultured brain slice applications. Here we present the capabilities of the MED64 Quad-II system, a novel medium-throughput MEA designed specifically for acute or cultured slice applications. We demonstrate the reliability and reproducibility of inducing LTP in acute hippocampal slices from 6-7 week old male ICR strain mice. First, I/O curves were obtained from CA1 in response to current driven stimulation delivered to the Schaffer collaterals in ACSF perfused (1-2 mls/min.) acute hippocampal slices heated to 32°C bath temperature. The high capacitance electrodes (55k pF) reliably produced greater than 1mV amplitude fEPSP at less than 30µA stimulus amplitude. The relatively large amplitude fEPSP in response to the relatively low stimulating current is due to the low impedance of the platinum black (10 kΩ at 1 kHz) and carbon nanotube (7 kΩ at 1 kHz) electrodes. Baseline amplitude and slope of the fEPSP was recorded for 15 minutes in response to stimulating current set to 30% of the current required to saturate the fEPSP amplitude. Following theta burst stimulation, amplitude and slope were monitored for an additional 60 minutes. We also explored the feasibility of chemically induced LTP and LTD using the MED64 Quad-II system. Furthermore, we demonstrate the throughput of the Quad-II system by acquiring extracellular recordings from four slices simultaneously, at 16 electrodes per slice. The results of this study can be applied to drug discovery, target validation, compound screening, *in vitro* mechanistic assays, as well as toxicology and pharmacology studies in acute brain slice applications.

Disclosures: M.S. Trujillo: A. Employment/Salary (full or part-time); Alpha MED Scientific. S. Yasuoka: A. Employment/Salary (full or part-time); Alpha MED Scientific.

Poster

540. Electrodes Arrays II

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Topic: G.04. Physiological Methods
**Support:** NIH 5R01DA034178-03

NSF CBET-1263785

2013 Alfred P. Sloan Research Fellowship

2013 Harvey L. Karp Discover Award

2014 McKnight Technical Innovations in Neuroscience Award

**Title:** A 3D neural probe with 1024 electrodes II: Dynamics and functional organization of reward circuitry

**Authors:** *J. L. SHOBE*1,2, L. D. CLAAR2, K. I. BAKHURIN2, S. PARHAMI2, S. C. MASMANIDIS2; 1UCLA, Irvine, CA; 2Neurobio., UC Los Angeles, Los Angeles, CA

**Abstract:** We used a newly developed 3D silicon microprobe to simultaneously record activity and functional interactions in the frontal cortex and several basal ganglia nuclei, structures that are known to support reward-guided behavior. Specifically, we conducted parallel recordings in the orbitofrontal cortex, anterior and posterior regions of the striatum, the globus pallidus, and the ventral tegmental area/substantia nigra nuclei with alert head-restrained mice performing a Pavlovian odor discrimination task. We conditioned mice on two trial types: 1) they learned to associate a specific odor with a reward (CS+ trial) and 2) they were presented with a different odor without reward (CS- trial). Our recordings revealed neural activity patterns that varied according to trial type. The relationship between the cortex and the basal ganglia structures was especially revealing. During the CS+ trials, the cortex had the lowest response to the reward-predicting cue and had the fewest number of neurons that discriminated between CS+ and CS- trials. However, relative to the basal ganglia, the activity in the cortex showed a dramatic increase in activity when we introduced surprise reward trials suggesting that it is tuned to detect unexpected events. Furthermore, we used principle component analysis (PCA) to visualize the neural dynamics between CS+/CS- and surprise trials revealing unique trajectories. For instance both CS+ and CS- trajectories shared a common pathway during the odor presentation, but shortly after odor offset CS- quickly diverged to its original state likely representing the rapid recall of a discriminatory representation. We also found interregional correlated firing patterns (using k-means clustering analysis) suggesting that discrimination behavior utilizes ensembles linked across the recording field. Interestingly these cue-discriminating cells may be predisposed to fire together through common wiring because we found a significant relationship between resting state functional connectivity and task-evoked correlations. Taken together, our observations demonstrate the capabilities of our 3D microprobe to simultaneously capture network dynamics at multiple scales relevant to understanding reward circuit function.

Poster

540. Electrodes Arrays II

Location: Hall A

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Topic: G.04. Physiological Methods

Support: ERC Advanced Grant “NeuroCMOS” AdG 267351
EU Marie Curie Initial Training Network (ITN) EngCaBra, 264417

Title: Tracking the activity of multiple individual neurons over one month in organotypic hippocampal slices grown on high-density multi-electrode arrays

Authors: *W. GONG1, J. SENČAR2, D. JÄCKEL1, D. BAKKUM1, A. HIERLEMANN1; 1BSSE, ETH Zurich, Basel, Switzerland; 2Fac. of Electrical Engin., Univ. of Ljubljana, Ljubljana, Slovenia

Abstract: The hippocampus is a well-characterized brain region for investigating neuronal plasticity, neurodegeneration, epilepsy and neurotoxicology, because of its relatively simple neuronal circuitry and cell types. Organotypic hippocampal slice cultures partially preserve the synaptic circuits and cytoarchitecture, while aspects of neuronal development can be observed in detail, beginning after slice preparation during the early postnatal days. In the current project, we established a method to cultivate organotypic hippocampal slices directly on high-density microelectrode arrays (HD-MEA) over long-term periods. The HD-MEA has an array area of 2x2 mm² containing 11,011 platinum electrodes at a pitch of 17 µm (U. Frey et al., 2009). Sagittal hippocampal slices (300 µm thickness) were obtained from newborn mice and affixed to HD-MEA chips with chicken plasma and thrombin. The slice cultures on the HD-MEAs were maintained in custom culturing chambers, designed to implement the roller tube method (B.H. Gähwiler, 1981). During the recordings, 126 electrodes can be arbitrarily selected for simultaneous recordings. Sequences of high-density configurations were used to record from the whole electrode array area. Spike amplitude and frequency on each electrode were analyzed to obtain comprehensive network activity maps. An unsupervised iterative spike sorting algorithm was developed, based on PCA and k-means clustering, in order to obtain the spike-triggered average extracellular waveforms across subsets of recording electrodes (“neuron footprints”). The slice cultures were recorded from for over 30 days in vitro. Footprints of individual neurons
only exhibited slight changes between consecutive days, which indicates that the position of the neurons remain relatively stable. Our results demonstrate that multiple individual neurons within a developing hippocampal network can be identified and that their activity can be tracked during long-term cultivation.


Poster

540. Electrodes Arrays II

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Topic: G.04. Physiological Methods

Support: BMBF 01 GQ 0814

Title: Implantable, yet adaptive computer-controlled multi-electrode positioning system for intracortical recordings in primates

Authors: *E. Ferre1, L. Suriya-Arunroi1, D. Hoehl2, U. Thomas2, A. Gail1,3,4; 1Cognitive Neurosci. Laboratory, Sensorimotor Group, German Primate Ctr., Goettingen, Germany; 2Thomas RECORDING GmbH, Giessen, Germany; 3Bernstein Ctr. for Computat. Neurosci., Goettingen, Germany; 4Fac. of Biol. and Psychology, Georg-August Univ., Goettingen, Germany

Abstract: Neuronal recordings with depth-adjustable microwire electrodes in non-human primates are a key method to investigate the neural basis of behaviors. Conventional micromanipulators for electrode positioning, however, are not chronically implantable and do not allow to record from many neurons simultaneously, limiting their use for brain computer interface applications or network analyses among populations of neurons. On the contrary, chronically implantable multi-electrode arrays allow recording from many neurons for a prolonged time, but electrodes are typically immovable. Hence, they do not allow optimizing the signal quality during and after implantation and are affected by tissue responses that progressively impair the transduced signal quality. This limits de facto the number of independent neurons that can be recorded over the lifetime of the implant. A third class of multi-channel recording methods makes use of chronically implanted matrices containing a higher count of individually movable electrodes to partially overcome these limitations. Yet, this latter technology does not provide ways to control electrode movements in a computerized fashion.
Instead, reposition the electrodes requires manipulation of manual actuators resulting in long-lasting interaction of the operator with the implant-holding animal. Here we demonstrate a chronically-implantable adaptive multi-electrode positioning (AMEP) system with detachable micro-positioning system for computerized depth-adjustment over several millimeters of each individual electrode after implantation. We show that by means of this semi-chronic 16 channel system we were able to record from many neurons simultaneously at variable depths from the cerebral cortex of a rhesus monkey. The system allowed recording of local field potential as well as single units while being able to reposition the electrodes in a computer-controlled fashion at micrometric precision and without interacting with the animal. Importantly the electrode-holding device is designed to remain within the recording chamber for a prolonged amount of time or can be used for acute recordings. The system is therefore paving the way towards chronic recordings with movable electrodes particularly suited for brain computer interface applications.

**Disclosures:**

**E. Ferrea:** None.

**L. Suriya-Arunroj:** None.

**D. Hochl:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Thomas RECORDING GmbH, Giessen, Germany.

**U. Thomas:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Thomas RECORDING GmbH, Giessen, Germany.

**A. Gail:** None.

**Poster**

**540. Electrodes Arrays II**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

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**Topic:** G.04. Physiological Methods

**Support:** NIH Grant

**Title:** Electrophysiological monitoring of neural stem cell differentiation

**Authors:**

*J. Collins*1,2, H. C. Wong1, J. Kohana1, M. G. Banuelos3, P. H. Schwartz2;

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**Abstract:** Statistical assessment of the electrophysiological characteristics of neural stem cell (NSC)-derived neurons through single cell and field potential recordings have been of great interest in stem cell research. Here, we describe a microelectrode array cell culture assay
allowing for the differentiation of NSCs on the electrodes and evaluation of the resulting neuronal population activity and responses. NSCs were allowed to reach confluence on the electrodes and differentiated in the presence of an overlay of cortical mouse glia. Electrophysiological characterization was carried out using multichannel microelectrodes in a well of volume ~1ml. Limited electrical activity was observed in 2 weeks of differentiation with two distinct types of signals across the cells. One type featured only hyperpolarization with a valley height of ~100 uV, and the other type included both positive and negative potential changes with magnitudes of ~100 and ~40 uV, respectively. After 2.5 weeks of culture there was a pronounced increase in activity. With applied stimulation, pulses with amplitudes of 0.5 mA, widths of 0.2 and 1 ms, and frequencies of 0.5 Hz increased the burst rate to 3.2 kHz. The spikes’ peak and valley heights varied from 30 to 125 uV. After 4 weeks of culture, the cells reached full maturity and there were consistent groups of spike trains as cells fired at faster rates of up to 3.5 kHz. In addition, these signals featured strong hyperpolarization as valley heights increased up to 1 mV for both lower (50 uA, 2ms, 0.5 Hz) and higher (0.5 mA, 0.2 ms, 0.5 Hz) levels of stimulation. Peak heights varied up to 125 uV, so these were not as prominent as the negative signals. Similar trends carried over to the fifth week of culture, where we also observed signals with valley heights of up to 1 mV for both spontaneous activity and with applied stimulation (0.5 mA, 0.2 ms, 0.5 Hz). By using this multielectrode array cell culture assay, we will statistically explore spontaneous and stimulus-induced population responses from neurons under different disease and drug conditions for high-throughput applications.

Disclosures: J. Collins: 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.; PI for a NIH Grant 5R43MH104170. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Ownership Interest with Biopico Systems Inc. H.C. Wong: 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.; CoInvestigator for NIH Grant 5R43MH104170. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Ownership Interest with Biopico Systems Inc. J. Kohana: 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.; Personnel in NIH Grant 5R43MH104170. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Ownership Interest with Biopico Systems Inc. M.G. Banuelos: B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; NIH Grant 5R43MH104170. P.H. Schwartz: 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.; PI of subaward in NIH Grant 5R43MH104170.

Poster

540. Electrodes Arrays II

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

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Topic: G.04. Physiological Methods

Title: Microchannel electrode arrays for regenerative peripheral nerve interface

Authors: *A. N. ZORZOS¹, B. MAIMON¹, R. RISO¹, M. CARTY³, S. TALBOT³, T. R. CLITES², H. M. HERR¹;
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Abstract: In an effort to establish high-resolution, chronic, scalable, and bi-directional communication with peripheral nerves, a novel micro-electrode array has been developed. The array is oriented in a “micro-channel” format and leverages the regenerative properties of peripheral nerve fascicles. The device was chronically implanted in ferret, rabbit, and rat models for recording and stimulation of motor, sensory, and mixed nerves respectively. To improve axon regeneration, a novel collagen scaffolding was incorporated into the design for the prevention of inflammatory-mediated losses. During the regeneration phase, electrophysiological activity was measured in parallel with mechanical stimulation thresholds and biomechanical dynamics to identify any associated functional deficits. Furthermore, a new immunofluorescence technique was employed to characterize axons by subtype within each channel (e.g., motor, sensory, sympathetic) as a way of associating peripheral nerve composition with biomechanical dynamics and electrophysiological activity.
Abstract: Introduction Transcranial electric stimulation (TES) is an emerging technique to non-invasively modulate brain function. However the spatiotemporal distribution of electric fields during TES remains poorly understood, and some question how much current actually reaches the brain. In this study we perform intracranial measurements of the electric field generated by each of two common TES modalities (transcranial direct current stimulation [tDCS], transcranial alternating current stimulation [tACS]) in epilepsy patients and a cebus monkey to investigate its spatial and temporal characteristics. Methods Patient recordings Four presurgical refractory epilepsy patients, with intracranially implanted electrodes participated in a single TES session. Two sponge electrodes (25cm2) were attached over the left and right temporal and a current of 1mA was applied for 2 min. We measured electric fields of tDCS and tACS with frequencies of 1Hz, 5Hz and 10Hz. Monkey recordings Three electrodes, with a total of 32 contacts (5mm apart) were permanently implanted through a skull incision over the left occipital cortex with posterior-anterior orientation. In multiple sessions intracranial EEG was recorded during TES. Small round stimulation electrodes (3.14 cm2, Ag/AgCl with conductive gel) were used in all sessions. We systematically varied the current strength to test for linearity of the conducting medium. To investigate the temporal course of electric fields during tACS and to test for possible frequency dependent effects on field strength or phase shifts, we parametrically varied the frequency of stimulation from 0.2 Hz to 150 Hz. Results We found a
linear relationship between the input current and the recorded intracranial voltage, thus indicating a linear resistive medium. Analysis of the frequency response indicated that power magnitude did not change with stimulation frequency. We observed only very small phase differences up to 3 degrees. We found measured potentials to be in accordance with predictions from computational models with spatially continuous varying potentials from the anode to the cathode for each montage. **Discussion** We conducted a comprehensive evaluation of the intracranial electric field during TES in both human patients and monkeys. Our results indicate that TES currents spread in a linear ohmic manner. Capacitive effects are if present very small. We did not observe frequency dependent attenuation of recorded signals. Our measurements can be useful for optimizing stimulation protocols and to understand physiological or behavioral effects from TES experiments.


**Poster**

**540. Electrodes Arrays II**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 540.13/CC29

**Topic:** G.04. Physiological Methods

**Support:** FDA-DARPA IAA 224-14-6009

**Title:** A test platform to evaluate the long-term safety and performance of peripheral nerve electrodes for brain-computer interface application

**Authors:** *S. VASUDEVAN, C. WELLE;

Food and Drug Administration, CDRH/OSEL/ Div. of Biomed. Physics, Silver Spring, MD

**Abstract:** Injuries causing limb loss or amputation can have deleterious effects on the quality of life. In the U.S. alone, there are ~1.6 million individuals suffering limb amputation. For this population, signals from the nervous system can be used to control advanced prostheses. Residual peripheral nerves after amputation offer safer and easier access for electrode implantation, as compared to brain. Studies show the availability of motor signals from peripheral nerves long after amputation and that these nerves can be stimulated to elicit sensory percepts (Rossini et al., 2010; Tan et al., 2014). This study aims to develop a test platform to evaluate the long-term safety and performance of electrodes implanted in the peripheral nerves.
We evaluated Floating Microelectrode Arrays (FMA) using bench and animal tests. Bench tests were performed by measuring impedance of the electrodes in PBS once a week for three weeks under dry (dried between measurements) and soaked (submerged in PBS throughout) conditions. For animal studies, electrodes were implanted in the rat sciatic nerve and connectors were secured using custom 3D-printed mounts. Electrode impedance was measured before and after implantation, until device failure. Motor function was evaluated using walking-track analysis and sensory deficits were assessed using Von Frey test. Electrodes were harvested for analysis using SEM and tissues were subjected to IHC. Bench tests showed minor fluctuations in impedance under dry conditions, while soaking caused drastic decrease. In animal studies, impedance of electrodes increased upon implantation and then stabilized. Electrophysiological recordings showed neural activity in ~25% of the electrodes, while the ability of an electrode to record over time was inconsistent between recording sessions. Between FMA and sham operated animals, there was no significant difference in motor function or sensory deficits. However, there was significant difference in sciatic and tibial function index between FMA group and uninjured animals at 1 wk. Devices failed between 6 and 13 wks, primarily due to lead wire breakage, although SEM revealed alterations to insulation and gaps between metal which may have contributed to loss of performance. Myelinated and unmyelinated axons around electrodes were observed using IHC. This regulatory science research study serves as a test platform to evaluate peripheral nerve interface technologies.

**DISCLAIMER:** The mention of commercial products, their sources, or their use in connection with material reported herein is not to be construed as either an actual or implied endorsement of such products by the Department of Health and Human Services.

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**Poster**

**540. Electrodes Arrays II**

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**Topic:** G.04. Physiological Methods

**Support:** European Union’s Seventh Framework Program (FP7/2007-2013) under grant agreement no. 600925 (NeuroSeeker)

**Title:** In vivo validation of a cylindrical 64-channel depth probe with a diameter of 800 µm

**Authors:** *F. POTHOF*¹, L. BONINI², M. LANZILOTTO³, A. LIVI¹, L. FOGASSI¹, G. A. ORBAN³, O. PAUL¹, P. RUTHER²
Abstract: Drug resistant focal epilepsy may be treated by localizing and resecting the epileptic focus. The precise pre-operative localization of the focus is based on stereoelectroencephalography (SEEG) probes implanted intracranially [1, 2]. The spatial resolution of this approach is however limited by the low number of recording sites, i.e. up to 18 electrodes, provided by commercial technologies. Here we report on the fabrication, characterization and \textit{in vivo} application of a 64-channel depth probe with recording sites of 35 \(\mu\)m in diameter. In order to validate the probe in the macaque posterior parietal cortex (PPC), the length was chosen to be 32 mm based on preliminary MRI scans of the monkey’s brain. The probe has an outer diameter of 800 \(\mu\)m, similar to clinically approved probes, and is realized by reshaping a planar layer stack made of two polyimide films and a patterned metallization sandwiched in-between [3]. The cylindrical probe geometry of the polyimide foil rolled inside a metallic mold is secured during the reshaping process using a dry adhesive. The probe is further stiffened by a polymeric refill resulting in a mechanical stiffness comparable to that of commercial devices. The probe was implanted manually in one monkey (Macaca mulatta), spanning the PPC from the inferior parietal convexity to the mesial parietal cortex, through a dedicated bone screw. The connectors interfacing the probe with the hardware-software platform from OpenEphys were protected by a custom-made recording chamber anchored to the skull. Already 24 hours after probe implantation we could record simultaneously well isolated single units activity (SUA) from 12 distinct sites. A modulation of local field potentials, multi-unit activity, as well as SUA was observed while the monkey performed various hand-arm motor tasks. The long-term recording stability demonstrated with the probes exceeds the usual time (1 week) needed for focal epileptic focus localization. References [1] J.M. Scarabin, \textit{Stereotaxy and Epilepsy Surgery}, John Libbey Eurotext ltd, 2012 [2] M. Cosu, et al., \textit{Neurosurgery}, 57(4), 2005, pp. 706-718 [3] F. Pothof, et al., \textit{Proc. IEEE EMBS Conf. 2014}, pp. 5244-5247


Poster

540. Electrodes Arrays II

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program# /Poster#: 540.15/CC31

Topic: G.04. Physiological Methods
Title: Carbon nanotube multi-electrode arrays for high sensitive extracellular measurements in cultured human iPSC derived neurons

Authors: *I. SUZUKI¹, N. MATSUDA¹, A. ODAWARA¹, M. FUKUDA², H. JIKO²; ¹Tohoku Inst. of Technol., Sendai, Miyagi, Japan; ²Alpha MED Scientific Inc., Osaka, Japan

Abstract: Multi-electrode arrays (MEAs) can be used for noninvasive, real-time, and long-term recording of electrophysiological activity in cultured neuronal network. Functional characteristics of human induced pluripotent stem cells (iPSC) derived neurons on their long-term spontaneous activity and drug responsiveness may be monitored by MEAs but many lack, cell affinity, sensitivity, signal-to-noise ratio (S/N), and durability. In this study, we describe the development of planar carbon nanotube (CNT)-MEA chips for high sensitive extracellular measurements in cultured human iPSC derived neurons. These CNT-MEA chips were fabricated by electroplating the indium-tin oxide (ITO) microelectrode surfaces. At 1kHz, the impedance of CNT electrodes with a diameter of 50 µm was approximately 8 kΩ. CNT electrodes exhibited both low impedance and high durability. Human iPSC derived neuron culture experiment reveal excellent biocompatibility and adhesion of CNT-MEA surface. In addition, action potential signals were recorded from human iPSC derived neurons with high S/N ratio and for > 9 months with long-term spontaneous activity. Drug responsiveness using synapse agonist and antagonist were also observed. Our CNT-MEA chips may be beneficial for clarifying the functions of human neuronal circuits and for drug screening applications.


Poster

540. Electrodes Arrays II

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 540.16/CC32

Topic: G.04. Physiological Methods

Title: The Encephalophone: A novel brain-computer music interface and cognitive rehabilitation device using conscious control of electroencephalogram (EEG)

Authors: *T. A. DEUEL¹,², J. PAMPIN⁵,², J. SUNDSTROM³, F. DARVAS⁴; ¹Swedish Neurosci. Inst., Seattle, WA; ²Ctr. for Digital Arts and Exptl. Media (DXARTS), ³Sch. of Music, ⁴Dept. of Neurosurg., Univ. of Washington, Seattle, WA; ⁵Sch. of Music, Unive, Seattle, WA
Abstract: A novel musical instrument was created using electroencephalogram (EEG) motor imagery to control a synthesized piano, and is herein named the Encephalophone. Alpha-frequency (8-12 Hz) signal power, originating from either posterior dominant rhythm (PDR) in the visual cortex or from mu rhythm in the motor cortex, was used to create a power scale which was then converted into a musical scale which could be manipulated by the individual. Subjects could then generate different notes of the scale by activation (event-related synchronization) or de-activation (event-related desynchronization) of the PDR or mu rhythms in visual or motor cortex, respectively. In a pilot study with 13 normal subjects, subjects were tested in their ability to hit target notes presented within a five-minute trial period. All 13 subjects were able to perform more accurately than a random note generator when using either visual cortex/PDR signaling or motor imagery/mu signaling. The Encephalophone's accuracy will be improved by training within the musical context, and has future applications as a novel musical instrument without requiring movement, as well as a potential therapeutic device for patients suffering from motor deficits (e.g. Amyotrophic lateral sclerosis, brainstem stroke, traumatic amputation).

little to no immune response, have the capacity to concurrently record from a large population of neurons, and demonstrate the ability to chronically record neural activity. To this end we have developed a 150 µm pitch, multi-electrode array, using carbon fibers (d=8.4 µm), which have been shown to be minimally damaging to the brain. To demonstrate the viability of this design as a chronic electrode technology we implanted 5 Long Evans rats with carbon fiber arrays using a poly(ethylene glycol) coating to assist in insertion. A subset of the animals implanted with carbon fiber arrays (n=2) and a separate cohort (n=3) were implanted with a planar silicon electrode (177 µm² site size, NeuroNexus Technologies) in the contralateral hemisphere’s motor cortex. Animal implant durations ranged from 3-5 months. Results from electrophysiology recordings show that on average 37% of carbon fiber electrodes (n=60 fibers) were able to detect unit activity for at least 3 months with an average amplitude of 203.1 µV over that time. In addition, units detected on the carbon fibers had an average SNR of 4.22 for the first 3 months. The chronically implanted silicon electrodes (n=5, 16 sites each) detected very few units, with an average amplitude of 95 µV during the 3 month implant period. The overall lack of detectable unit activity on the silicon electrodes may be partially attributable to the small site size. Histology intensity values were calculated by averaging the fluorescent signal within the first 50 µm of the implant site(s). This value was then normalized by the control value which was calculated by averaging together the intensities of each corner (500 µm x 500 µm) of the image. Analysis from one rat showed the reactive response to the carbon fiber arrays was minimal with a normalized astrocytic intensity of 2.29 and microglial intensity of 0.75. Reactive response to the silicon electrodes was characterized by the formation of a scar with an astrocytic intensity of 8.68 and microglial intensity of 2.62. This work has demonstrated the ability of carbon fiber electrodes to chronically record unit activity in the rat motor cortex out to 16 weeks with minimal scarring. The units detected were of large amplitude and showed a high SNR. Future work will seek to improve array packaging to allow for even higher density arrays with higher channel counts that will allow us to record from all neurons in a given cortical layer.


Poster

540. Electrodes Arrays II

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 540.18/CC34
**Title:** Carbon fiber based microelectrode array for intracortical neural recording

**Authors:** *Y. LEE*¹, Y. LIM², S. HWANG¹, H. YOO¹, S. JUN¹,³; ¹Ewha Womans Univ., Seodaemun-Gu, Seoul, Korea, Republic of; ²Ctr. for Robotics Research, Robotics and Media Institute, Korea Inst. of Sci. and Technol., Seoul, Korea, Republic of; ³Dept. of Brain and Cognitive Science, Ewha Womans Univ., Seoul, Korea, Republic of

**Abstract:** Microelectrode arrays are commonly used for electrophysiological recording from individual neurons to study neural pathways in the brain or to develop brain-computer interface. However, the long-term recording is still limited due to the tissue reaction around the implanted microelectrodes even though the chronic neural recording is becoming more and more essential for long-term applications. It is believed that the geometry of the electrode such as the size is the most critical component which can determine the degree of the chronic tissue damage. In this study, in order to reduce the reactive tissue response, carbon fiber-based microelectrode arrays are developed. Since the diameter of the carbon fiber (7 µm in diameter) is much smaller than the conventional micro-wires, it is expected that the carbon fiber neural probe is appropriate for the long-term neural recording. We introduce the multi-channel microelectrode array whose channels are composed of multiple carbon fiber bundles and show the preliminary neural responses recorded in motor cortex of anesthetized rat. While each carbon fiber is too flexible to be inserted in the rat brain, the bundle of carbon fibers can sustain each other to insert the electrode into the brain. Each neural signal from a carbon fiber is merged into a single electrical channel and the individual neural spikes can be identified via the spike sorting afterwards.

**Disclosures:** Y. Lee: None. Y. Lim: None. S. Hwang: None. H. Yoo: None. S. Jun: None.

**Poster**

540. Electrodes Arrays II
Title: Differential impact of anesthetics on real-time electrochemical recordings of glutamate neurotransmission in the rodent brain

Authors: *T. COLBURN;
Univ. of Arizona, Phoenix, AZ

Abstract: Across physiological recording paradigms, urethane is commonly used as a minimally confounding anesthetic. A paper published by Hara and Harris implicated urethane in affecting multiple neurotransmitter systems, bringing into question the utility of urethane in neurophysiological recordings. Isoflurane is commonly used for neurological studies due to minimal side effects, rapid post-operative recovery, and a high level of control over its administration. We test the hypothesis that isoflurane is a viable alternative to urethane for in vivo amperometric recordings of glutamate neurotransmission in the rodent brain. We used novel glutamate-selective microelectrode arrays (MEAs) to measure glutamate clearance kinetics in the cortex and thalamus of anesthetized adult male Sprague-Dawley rats. In separate cohorts of animals, isoflurane was delivered at 2% (in O2) via nose cone throughout the recordings or urethane (~1.25g/kg) was given as serial intraperitoneal injections until loss of pedal reflex was achieved. In vivo local applications of glutamate and potassium chloride were administered within each region of interest in order to assess real-time glutamate dynamics. Preliminary results indicate that potassium-chloride evoked release of glutamate tends to be greater in isoflurane anesthetized rodents in comparison to urethane, regardless of anatomical loci. These data suggest that the choice of anesthetic in anesthetized recordings of real-time neurotransmission can influence glutamate release and uptake dynamics, confounding comparisons between experimental paradigms.

Disclosures: T. Colburn: None.
Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 540.20/CC36

Topic: G.04. Physiological Methods

Support: CIHR, NSERC, FRQ, Weston Brain Institute, Michael J. Fox Foundation for Parkinson's Research, Alzheimer's Society, Brain Canada

Title: Deep brain stimulation in mice using magnetic resonance imaging-compatible carbon electrodes

Authors: *D. R. GALLINO, V. KONG, G. DEVENYI, A. MATHIEU, M. CHAKRAVARTY; Cerebral Imaging Ctr., Douglas Mental Hlth. Univ. Inst., Verdun, QC, Canada

Abstract: Deep brain stimulation (DBS) is used to deliver chronic high-frequency electrical stimulation to brain circuits that are compromised by neuropsychiatric disorders. DBS has been used effectively in the treatment of Parkinson’s disease, and there are several new investigations of DBS in the context of other disorders (e.g. major depression, Alzheimer’s disease). Preclinical longitudinal magnetic resonance imaging (MRI)-based investigations of novel targets are hampered by the use of traditional non-MR compatible electrodes. Here we investigate the feasibility of using carbon-based electrodes for delivering DBS in mice with respect to MR compatibility, biocompatibility, and electrical properties. Monopolar electrodes were constructed using carbon-fiber rods of 0.25 and 2 mm diameters, bound with conductive carbon epoxy and insulated with polyvinyl alcohol. Electrodes were implanted in C57BL/6 male mice, perpendicularly to the skull plane at bregma, +/- 1 mm laterally and at a depth of 3.75 mm to target the body of the fornix. Animals were imaged in a 7T Bruker USR magnet (20 cm bore) using a TrueFISP 3D acquisition with 100 µm isotropic voxels. Monophasic stimulation was delivered at 120 Hz, 200 µA, 1 V with pulse width of 100 µs. Mean and standard deviation of electrode resistance was 1379 and 248 Ω respectively. Neither electrode implantation nor stimulation caused noticeable adverse side-effects in mice. Stimulation at 200 µA required 1 V, less than comparable metal electrodes, and indicating a total circuit resistance of ~5 kΩ. High resolution MR images were generated in under 90 min and contained no noticeable artifacts generated by the electrodes. The lack of artifacts allowed for accurate confirmation of electrode placement and imaging of the surrounding fine structures (Fig.1). We believe that these images are suitable for further structural analyses. Our findings suggest carbon fibre to be an appropriate replacement material for metal in DBS electrodes due to its high conductivity, biocompatibility and the absence of MRI acquisition artefacts.
Disclosures: D.R. Gallino: None. V. Kong: None. G. Devenyi: None. A. Mathieu: None. M. Chakravarty: None.

Poster

540. Electrodes Arrays II

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 540.21/CC37

Topic: G.04. Physiological Methods

Support: NINDS Intramural Program

Title: Standardized regions of interest for population analyses using electrocorticography (ECoG)

Authors: *J. B. COCJIN*¹, S. R. DAMERA², Z. S. SAAD³, S. K. INATI³, K. A. ZAGHLOUL¹; ¹Surgical Neurol., ²Office of the Clin. Director, NINDS, Bethesda, MD; ³Georgetown Univ., Washington, DC; ⁴Scientific and Statistical Computing Core, NIMH, Bethesda, MD

Abstract: The high spatiotemporal resolution of electrocorticography (ECoG) renders it invaluable for characterizing focal activity such as that associated with recurrent seizures or cognitive functioning. Unlike other recording modalities such as fMRI or scalp EEG, ECoG lacks standardized means for attributing electrode activity to spatially similar regions across subjects. Current methods either limit analyses within the confines of presumably relevant anatomical structures, ignoring possible effects in other areas of electrode coverage, or conduct averages over broad regions such as gyri, reducing the spatial specificity of the analyses. Here, we attempt to balance spatial coverage and specificity appropriate for ECoG by defining regions-
of-interest (ROIs) as the nodes of low-density standard pial meshes generated in SUMA, which have indices of similar location across subjects. We first localize electrodes to an envelope about a reconstructed pial surface via a semi-supervised projection algorithm. We then assign high-density standard mesh nodes to each electrode through a combination of capture within the electrode's model volume and geodesic growth along the mesh surface. Finally, we use a nearest neighbor mapping to gather high-density nodes into the low-density ROIs. We demonstrate that group analysis across varying mesh densities for a motor task yields similar regions of activation. We expect that the adoption of standardized low-density ROIs that match the spatial properties of ECoG has the potential to yield insights not possible with current mapping schemes.


Poster

540. Electrodes Arrays II

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

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Topic: G.04. Physiological Methods

Support: NIH 5R01DA034178-03

NSF CBET-1263785

2013 Alfred P. Sloan Research Fellowship

2013 Harvey L. Karp Discover Award

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Title: A 3D neural probe with 1,024 electrodes I: Probe design and development

Authors: *L. D. CLAAR*, J. L. SHOBE, S. PARHAMI, K. I. BAKHURIN, S. C. MASMANIDIS;

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Abstract: Our goal was to develop an electrophysiological recording system capable of monitoring neural data at multiple scales in the mouse brain. Existing recording technologies
have a limited ability to interrogate local microcircuit activity as well as systems-level dynamics spanning multiple brain regions in parallel. To this end, we developed a three-dimensional (3D) electrode array via a multilayered assembly method to bond together four microfabricated planar silicon microprobes that are precisely aligned using motorized micromanipulators to target different anatomical regions of the mouse brain. The 3D array contains a total of 1,024 simultaneously accessible recording sites, which are densely patterned across multiple silicon prongs. For this work, we targeted key regions implicated in reward processing and movement (orbitofrontal cortex, striatum, globus pallidus, ventral tegmental area, and substantia nigra). To minimize the device’s external wiring, we developed multiplexed head stage modules that coupled with each layer of the assembly. To verify the functionality of the recording system we performed acute recordings in awake, head-fixed mice performing a Pavlovian odor discrimination task (see linked poster by Shobe et al). We developed a custom single-unit spike sorting algorithm designed for high electrode density recordings that can distribute spike sorting tasks on multiple computers to reduce computation time. Recordings yielded an average of over 300 well-isolated units across the electrode array. With the precise, micrometer-level control we have over the relative positioning between each electrode in three dimensions, these 3D microprobes can be designed to combinatorially study a large number of brain structures. The ability to record from multiple areas in parallel presents new opportunities to address important questions regarding the multi-scale functional organization of brain circuits that exists in health and disease.


Poster

540. Electrodes Arrays II

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 540.23/CC39

Topic: G.04. Physiological Methods

Support: AMED Grant 15mk010429h0202

Title: Induction and characterization of synaptic transmission induced synchronized population bursts of the induced pluripotent stem cell-derived neurons

Authors: *N. MIYAMOTO, K. SAWADA;
EISAI Co., Ltd., TSUKUBA, Japan
Abstract: Many drugs have been reported to cause seizures. It has been reported that the causes of drug-induced seizures are GABA\(_A\) antagonism, GABA\(_B\) agonism, adenosine antagonism, and enhanced excitation through NMDA in the neurons. So far, there is no good \textit{in vitro} assay system for predicting drug-induced unexpected seizure-risks. Spontaneous neuron activity recordings by multi-electrode array (MEA) system from networks of cultured neurons could be a good risk evaluation system for such drug-induced seizure events [1]. It was reported that long-term electrophysiological activity and pharmacological response of human induced pluripotent stem cell (hiPSC)-derived neurons were accelerated by co-culture with rat astrocytes [2]. In this study, we observed time course generation of population burst spikes from iCell neurons with conditioned medium of mouse primary astrocytes by MEA system. Humoral factor(s) from mouse primary astrocytes was sufficient to generate synchronized population burst spikes in the iPSC-derived neurons. GABA antagonism enhanced the periodic synchronized burst spikes in a dose-dependent manner. P/Q-type and N-type calcium channel blockers eliminated the periodic synchronized burst spikes, suggesting that the burst spikes are mediated by synaptic transmission. We concluded that the observed astrocyte-induced population bursts by MEA system are mediated by synaptic transmission and the periodic synchronized population burst signals could be a good prediction marker of GABA\(_A\) antagonism. [1] E. Biffi et al., PLoS ONE 8 (2013) e83899. doi:10.1371. [2] A. Odawara et al., Biochem. Biophys. Res. Commun. 443 (2014) 1176-1181. This research is partially supported by the grants for iPS Non-clinical Experiments for Nervous System (iNCENS) project in Research Grants on Regulatory Science of Pharmaceuticals and Medical Devices from Japan Agency for Medical Research and development, AMED.

Disclosures: N. Miyamoto: None. K. Sawada: None.

Poster

540. Electrodes Arrays II

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 540.24/CC40

Topic: G.04. Physiological Methods

Support: EuroSPIN Erasmus Mundus programme and NCBS/TIFR

Istituto Italiano di Tecnologia

7th Framework Programme for Research of the European Commission, under Grant agreement no 600847: RENVISION project of the Future and Emerging Technologies (FET) programme Neuro-bio-inspired systems (NBIS) FET-Proactive Initiative
Title: Automated spike sorting across electrodes in large-scale recordings from the mammalian retina

Authors: *M. SORBARO SINDACI*1, G. HILGEN2, S. PIRMORADIAN1, I. KEPIRO1, S. UULLO3, O. MUTHMANN4, L. BERDONDINI1, D. SONA3, E. SERNAGOR2, M. H. HENNIG1; 1Inst. for Adaptive and Neural Computation, Sch. of Informatics, Univ. of Edinburgh, Edinburgh, United Kingdom; 2Inst. of Neurosci., Univ. of Newcastle, Newcastle-upon-Tyne, United Kingdom; 3Inst. Italiano di Tecnologia, Genova, Italy; 4Natl. Ctr. for Biol. Sci., Bangalore, India

Abstract: Microelectrode arrays (MEAs) are a powerful tool, capable of sampling, at high frequencies, the electrical potential at hundreds or even thousands of locations simultaneously, and are widely used to record the responses of large neural populations. Currently, spikes observed by each electrode are sorted according to their waveform, whose shape shows variations across neurons; significant challenges, however, arise when working with high-density MEAs, where individual neurons may be recorded simultaneously by multiple neighbouring electrodes. Waveform clustering, moreover, often needs an amount of human intervention that makes it highly time-consuming for large arrays, and shows poor performances at sampling frequencies below 10-20 kHz. We integrate this approach with a novel technique, based on the pair of spatial coordinates associated to each spike by interpolation across neighbouring electrodes. Simultaneous events observed on different channels are associated to a point in space by comparing the amplitudes measured by each channel. Events are found to be mostly grouped in well-localised units, which we believe correspond to single neurons and we separate thanks to the Mean Shift clustering algorithm applied to spatial and waveform dimensions simultaneously. Both position and shape of an event can also be used to classify it as noise or signal. Our results show that this method -- applied to 4096-electrode MEA recordings from the mouse retina -- avoids the duplication of units observed by multiple electrodes and sorts spikes better than the sole principal component analysis of the waveforms. Furthermore, it gives information, at a resolution higher than that of the array grid, on the location of the units. The comparison with micrographs of green fluorescent protein-labelled neurons confirms the correct localisation and clustering of spikes from single neurons.
Figure: the two clusters shown are distinct in location (the first is centred on an electrode, the second between electrodes) and, consistently, show different spike shapes. Electrode spacing is 42 µm.


Poster

540. Electrodes Arrays II

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 540.25/CC41

Topic: G.04. Physiological Methods

Title: Construction of two-site multi-channel optrode system in freely moving mouse

Authors: *Y. TANG;
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Abstract: Objective Optogenetics technology provides an opportunity to control the activity of neurons for exciting or inhibiting the brain networks. Real-time observing and recording the neurons’ activity in the illuminated and related brain region become critical and powerful for exploring the principle of brain works. Here, we try to construct a two-site multi-channel optrode
system in freely moving mouse. **Methods** We integrated the optogenetic stimulation technology and multi-channel recording technology, to form a two-site multi-channel optrode system. This system contains two bundles of electrodes, one bundle combines a φ230µm multimode optical core for optical illumination and 8 tetrodes surrounding the optical fiber for neuron activity recording, the tips of 8 tetrodes are 0.5-0.7mm longer than the end of optical fiber. All of the electrode tips were plated with platinum(chloroplatinic acid solution) to a final impedance of 0.5-0.8MΩ. Both of bundles can be driven independently and have a range of 5mm for moving, the optrode advanced 300µm once the screw driver turn a circle. Thus, the optrode system can illuminate and record in every depth in a certain two-site at the same time. **Results** The two-site optrode system got the action potentials credibly from the illuminated brain region and the related area at the same time. **Conclusion** The two-site optrode system is an appropriate option for research in freely moving mouse.

**Disclosures:** Y. Tang: None.

**Poster**

541. Novel Assays

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 541.01/CC42

**Topic:** G.04. Physiological Methods

**Support:** NIH R01 NS70872

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The Grainger Foundation

**Title:** Development of a wirelessly controlled multichannel neurochemical sensor and neurostimulator: WINCS Harmoni

**Authors:** *K. H. LEE¹, E. K. ROSS², J. K. TREVATHAN³, M. P. MARSH², R. A. PHILPOTT¹, J. S. HUMBLE³, C. L. FELTON⁴, B. K. GILBERT⁵, C. J. KIMBLE⁴, M. B. MCINTOSH⁴, K. R. KRESSIN⁴, J. B. BOESCHE⁴, D. R. EAKER⁴, J. L. LUIJAN⁵, A. J. BIEBER², S.-Y. CHANG², K. E. BENNET⁶;

**Abstract:** Introduction: Deep brain stimulation (DBS) is an effective neurosurgical treatment for Parkinson’s disease and essential tremor, and it is now being evaluated for treatment of psychiatric disorders. Despite its clinical success, there is still only a limited understanding of the therapeutic mechanisms behind DBS. Attempts to elucidate these mechanisms by measuring electrophysiological activity have thus far been inconclusive. We hypothesize that understanding the underlying therapeutic mechanism of DBS will require a combination of electrophysiological and electrochemical analysis. Since electrophysiological measuring techniques are well established in the literature, we developed the Wireless Instantaneous Neurochemical Concentration Sensing (WINCS) Harmoni system to characterize stimulation-evoked neurochemical responses. WINCS Harmoni can modulate neuronal activity in real-time response to neurochemical events. Method: WINCS Harmoni employs fast-scan cyclic voltammetry (FSCV) to characterize the neurochemical events during the application of DBS. WINCS Harmoni incorporates a microcontroller, Bluetooth transceiver, configurable and synchronizable isolated neurostimulator, and a custom four-channel integrated circuit for interleaved neurochemical and electrophysiological measurements, all powered by a pair of batteries. PC-based software provides real-time control of stimulation, data acquisition, and data visualization. Here, we examine WINCS Harmoni’s efficacy in vivo in the rat, pig, and non-human primate. We stimulated and recorded neurochemical measurements in parallel using a carbon fiber microelectrode (CFM) implanted in the striatum. FSCV was conducted by applying a series of pyramidal voltage waveforms to the CFM and measuring the currents produced by the resulting electrochemical activity. Results: In this proof-of-principle study, WINCS Harmoni successfully evoked and detected striatal dopamine release by DBS in the rat, pig, and the non-human primate. Notably, the synchronization of stimulation with interleaved FSCV scans eliminated the stimulus artifact that would have otherwise obscured the neurochemical measurements. Importantly, we were able to record FSCV data from four channels simultaneously during DBS with stimulation provided by WINCS Harmoni. Conclusions: WINCS Harmoni represents a major improvement in electrochemical sensing for neuromodulation control. The ability to simultaneously record electrochemical events and translate them into stimulation programming changes in real time will be a powerful tool toward closed-loop DBS.


**Poster**

541. Novel Assays

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM
Title: Real-time functional ultrasound imaging of brain activity on freely moving rats during active tasks

Authors: *A. URBAN*¹, D. CLARA², M. GUILLAUME², B. CLÉMENT², M. EMILIE³, M. GABRIEL²;
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Abstract: Innovative imaging modalities are required to investigate the complex relationship between brain activity and behavior in freely moving animal. Functional ultrasound (fUS) is a recent strategy for recording cerebral blood volume (CBV) dynamics in the whole brain but has so far only been used in head-fixed and anesthetized rodents. Here a fUS device was designed for performing tethered brain imaging in freely moving rats based on a miniaturized ultrasound probe and a custom designed ultrasound scanner. CBV changes were monitored in various behavioral states such as quiet rest, after whiskers or visual stimulations and also in a food-reinforced operant task. fUS imaging in a freely moving condition was fully efficient to decode brain activity in real-time based on analysis of CBV during reward collection. These results support the viability of fUS in future minimally invasive brain-machine interfaces. Figure Legend: Miniaturized device for functional ultrasound in freely moving rats. (a) Miniaturized ultrasound probe (b) CAD drawing of the magnetic head fixation system. The head plate (purple) is surgically implanted on the skull with 6 screws. The probe holder (brown) allows quick connections and disconnections of the probe using magnets (yellow). (c) Pictures of the rat at rest or during fin-fUS. A magnetic head shield protects the brain between imaging sessions. The probe is inserted in the probe holder and then connected to the head plate during experiments. (d) Coronal view of the imaging plane. The red square indicates the field of view of the probe allowing cortical and subcortical imaging of the hemodynamic signal. Barrel field (BF) cortex, motor (M) cortex, thalamus (TH) and hippocampus (HPC). (e) CBV images in freely moving conditions with the probe positioned on the left (top left, red square) or the right hemisphere (top right). Composite image of the entire brain after merging of left and right CBV images. Scale bars, 5 mm.

Poster

541. Novel Assays

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 541.03/CC44

Topic: G.04. Physiological Methods

Support: NIH Grant P01 AG00538

Title: Single-synapse analysis of long-term potentiation by flow cytometry

Authors: *G. A. PRIETO, B. H. TRIEU, G. LYNCH, C. W. COTMAN;
Inst. for Memory Impairments and Neurolog. Disorders, Irvine, CA

Abstract: Long-term potentiation (LTP), a lasting increase in the efficiency of synaptic transmission, was first recorded in a cortical-hippocampal circuitry and has been studied for decades using electrophysiological protocols for stimulation and recording. While in vivo and in vitro recordings have provided valuable information on LTP underlying mechanisms, the study of LTP would benefit from a simplified system intermediate between in vivo and in vitro approaches. Here we describe a novel approach to study LTP. This method focuses on the insertion of AMPA (α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptors
(AMPAR) into the post-synaptic surface, the essential event for the potentiation of synaptic transmission. Our approach consists of chemical LTP stimulation (cLTP) directly in fresh hippocampal synaptosomal fractions, immunofluorescence-labeling and flow cytometry analysis. Accordingly, we named this approach ‘Fluorescence Analysis of Single-Synapse Long-Term Potentiation’ (FASS-LTP). Fluorescence analysis after cLTP induction (45 min) identified an increased proportion of potentiated synapses (GluR1+) over basal levels, demonstrating that FASS-LTP detects activity-dependent plasticity in synaptosomes. Time course analysis revealed that the early (15 min) increase in the proportion of GluR1+ synaptosomes after cLTP stimulation is sustained up to 75 min, thus resembling the long-lasting increase in excitatory postsynaptic potentials (EPSPs) in hippocampal slices after electrical stimulation. We found that the NMDA receptor (NMDAR) antagonist AP5 blocks the cLTP-induced increase in GluR1+ synaptosome level. Finally, we evaluated cLTP response at 15, 25 and 45 min in young (3-4 months) and aged (12-15 months) 3xTg mice, a well-known model of Alzheimer’s disease (AD). While young 3xTg mice exhibited normal GluR1+ levels after chemical stimulation, cLTP response was utterly absent in aged 3xTg mice, a profile consistent with electrophysiological recordings in 3xTg showing intact LTP at 3-4 months but significant deficits in 6-7 month old mice26. Overall, these data demonstrate that FASS-LTP is a sensitive approach to study synaptic plasticity.


Poster

541. Novel Assays

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 541.04/CC45

Topic: G.04. Physiological Methods

Support: NINDS T32NS048005

MGH/ECOR Scholar’s Fund

Title: OpBox: Open-source development of customized and cost-effective hardware and software for behavioral neurophysiology

Authors: *B. F. COUGHLIN¹, B. E. SHANAHAN¹, G. PIANTONI¹,², S. S. CASH¹,², E. Y. KIMCHI¹,²;
¹Massachusetts Gen. Hosp., Boston, MA; ²Harvard Med. Sch., Boston, MA
Abstract: Open-source technologies, made available to use and modify without licensing or restrictions on use, is having a major impact on the development of laboratory software and hardware. The rise of technologies such as 3D printers has spurned the growth of the "maker" community, which seeks to build and adapt equipment from existing designs for customized purposes, rather than use less-than-ideal equipment that may not cover every need. A growing number of labs, including our own, are adopting this movement, allowing not only for customization of equipment, but also for collaborative improvements in design deemed most useful by the field. We have developed a modular, open-source behavioral neurophysiology system, collectively called OpBox. We have integrated several open-source technologies spanning the range from hardware to software: 1) 3D desktop printing, allowing us to model, fabricate, and use devices within the same day, as well as share them digitally so distant labs can have the same hardware to within µm of precision; 2) embedded microcontrollers such as Arduinos which allow for interfacing hardware and software via printable electronic circuit boards; and 3) multithreaded software to process multiple data streams concurrently in real-time. Using these three technologies we have developed a behavioral neurophysiology system for rodents called OpBox, capable of performing operant conditioning, tracking of locomotor activity, recording of electrophysiological activity, and video recording. We have dramatically cut costs down by up to an order of magnitude through a modular design which can be cheaply modified or replaced part-by-part as deemed necessary. This also allows for better collaboration with other labs performing similar experiments. We have created an online repository for our designs and how-to tutorials so that the broader community download and modify our designs/code. While there is a learning curve to open-source development, as more labs adopt these methodologies, there is a greater likelihood of finding designs/code that already fulfill customized needs or need only minor modifications to do so. Overall, open-source systems like OpBox promise to have tremendous impact on the practice of neuroscience research.

Disclosures:  
B.F. Coughlin: A. Employment/Salary (full or part-time); Massachusetts General Hospital.  
B.E. Shanahan: A. Employment/Salary (full or part-time); Massachusetts General Hospital.  
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Poster  
541. Novel Assays  
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Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM  
Program#/Poster#: 541.05/CC46
Abstract: Transcranial focused ultrasound (tFUS) is a highly promising tool for non-invasive neuromodulation. Recent studies have shown the potential for modulating and inducing sensations in the human somatosensory cortex (Legon et al. 2014, Lee et al. 2015). However, the mechanisms for how ultrasound affects neurons and what parameters are critical are unknown. Human studies have shown large variability within subjects, and it is very difficult to optimize parameters in humans. A refinement of our understanding is needed to apply ultrasonic neuromodulation in humans in safe, systematic manner. Many of the studies so far in vivo and ex vivo in animal models have utilized electrophysiological means or coarse imaging methods (BOLD, PET) requiring intensive labor with invasive probes that could affect how ultrasonic energy interacts with neurons, or offering poor spatial and temporal time scales. Calcium imaging of neural activity provides a non-invasive means of studying large populations of neural activity with high temporal and spatial resolution. Widefield imaging can provide a quick readout of population level activity and provide information about the spatial pattern of neuromodulation, while two-photon imaging can give cellular level resolution activity within an intact and unperturbed neuronal network. Here, we develop a system that allows easy optical means of observing neuronal responses to ultrasonic neuromodulation in transgenic mice with widespread cortical GCaMP6s expression and describe results from our studies.

Disclosures: T. Sato: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Thyne Inc.. F. Consulting Fees (e.g., advisory boards); Thyne Inc.. D. Tsao: None.
Title: Improved sensitivity and measurement stability of fast scan cyclic voltammetry measurements using the ‘elastic net’

Authors: H. LONG1, K. T. KISHIDA2, J. P. WHITE2, *R. J. MORAN2, T. LOHRENZ2, P. PHILLIPS3, P. DAYAN4, P. R. MONTAGUE2;

Abstract: Dopamine and serotonin are two neuromodulators implicated in a wide range of human psychiatric conditions. In vivo monitoring of these neuromodulators in model organisms and humans during ongoing behavior and decision-making will provide invaluable information about the role these neurotransmitters play in human cognition and mental health. Fast scan cyclic voltammetry (FSCV) on carbon fiber microelectrodes permits in vivo measurements of electrochemically active neurotransmitters such as dopamine and serotonin with sub-second temporal resolution. Current state-of-the-art approaches for determining the chemical identity and concentration from background subtracted cyclic voltammograms include the use of qualitatively determined oxidation peaks of relevant neurochemicals or waveform analysis using principal components regression (PC-regression). These approaches work well when chemical concentrations are high and background subtracted cyclic voltammograms result in qualitatively recognizable waveforms; however, at lower concentrations or in more complex chemical environments these methods have shown limitations. We employ a modern machine learning approach, the ‘elastic net’ (EN), to train multivariate penalized linear regression models for estimating and discriminating dopamine and serotonin concentrations from FSCV measurements. We compare the performance of the EN-based, PC-regression, and univariate approaches. We use in vitro measurements of prepared dopamine solutions to reproduce known limitations of oxidation peak and PC-regression based approaches. In contrast, we show that our EN-based procedure overcomes these issues, and offers more sensitive and stable FSCV-based estimates of dopamine and serotonin levels. Further, we use the EN-based model trained on in vitro data to reanalyze previously published FSCV measurements made in the striatum of freely moving animals and estimate dopamine levels evoked by electrical stimulation of the medial forebrain bundle.


Poster

541. Novel Assays

Location: Hall A
**Title:** *In vitro* assessment of biased signaling: a duplex assay approach to detect functional selectivity of 5-HT2C agonists

**Authors:** *V. LAKICS, B. RAUPRICH, M. H. BAKKER, A.-L. RELO, H. MACK, A. HAUPT, W. BRAJE, G. C. TERSTAPPEN, K. DRESCHER;* Abbvie Deutschland GmbH and Co. KG, Ludwidshafen, Germany

**Abstract:** Agonists of seven-transmembrane receptors often do not activate all linked signaling pathways to the same extent, but rather differ in their ability to signal through G-proteins or activate other signaling pathways. This phenomenon, termed biased signaling or functional selectivity, offers possibilities to design novel drugs with potentially better efficacy or therapeutic index. 5-HT2C receptor agonism is a promising mechanism to target in multiple neurological conditions, like drug abuse and various neuropsychiatric conditions, including schizophrenia. In this study, we have developed a novel approach for detecting functional selectivity in a series of 5-HT2C agonists and have also characterized these compounds. To reliably detect biased signaling, a duplex assay has been set-up, first measuring calcium-mobilization, then assessing beta-arrestin recruitment in the very same U2OS cells expressing the human 5-HT2C receptor. Performing both readouts from the same cells significantly decreased the variability of our measurements and allowed for a more reliable detection of bias by our agonists. Using these two readouts, we have compared the *in vitro* potency of over 50 selected 5-HT2C receptor agonists to that of a reference agonist, serotonin. No beta-arrestin preferring compounds were found, but several 5-HT2C agonists with G-protein signaling bias were identified. The well described selective 5-HT2C reference agonists, vabicaserin, lorcaserin and CP-809,101, showed no detectable bias in our system. G-protein-prefering compounds were inactive in an *in vitro* receptor internalization assay, while non-biasing agonists were capable of inducing beta-arrestin-mediated internalization. So far, no differential *vivo* activity of our biased 5-HT2C agonists was detected, using the amphetamine-induced hyperactivity assay in mice - additional *in vivo* assays are currently being applied to characterize these compounds. Taken together, our duplex cell-based assay is well-suited to identify 5-HT2C agonists, preferentially signaling through G-protein vs. beta-arrestin. The principle of our approach can easily be extended to other seven-transmembrane receptors to identify biased agonists within a specific lead series, increasing diversity and potentially resulting in better profiles for drug candidates.

**Disclosures:** All authors are employees of AbbVie. The design, study conduct, and financial support for this research was provided by AbbVie. AbbVie participated in the interpretation of data, review, and approval of the publication.
Disclosures:  V. Lakics: A. Employment/Salary (full or part-time); AbbVie Deutschland GmbH & Co KG. B. Rauprich: A. Employment/Salary (full or part-time); AbbVie Deutschland GmbH & Co KG. M.H. Bakker: A. Employment/Salary (full or part-time); AbbVie Deutschland GmbH & Co KG. A. Relo: A. Employment/Salary (full or part-time); AbbVie Deutschland GmbH & Co KG. H. Mack: A. Employment/Salary (full or part-time); AbbVie Deutschland GmbH & Co KG. A. Haupt: A. Employment/Salary (full or part-time); AbbVie Deutschland GmbH & Co KG. W. Braje: A. Employment/Salary (full or part-time); AbbVie Deutschland GmbH & Co KG. G.C. Terstappen: A. Employment/Salary (full or part-time); AbbVie Deutschland GmbH & Co KG. K. Drescher: A. Employment/Salary (full or part-time); AbbVie Deutschland GmbH & Co KG.

Poster

541. Novel Assays

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 541.08/CC49

Topic: G.04. Physiological Methods

Support: NIH 1DP2-EB018657

NIHM R01MH101218

ARO MURI W911NF-12-1-0594

Title: Nanomechanical characterization of synapses in live hippocampal neurons via torsional harmonic atomic force microscopy

Authors: *J. YANG¹, N. MANDRIOTA¹, J. JONES¹, D. KIM¹², R. LEFORT³, R. YUSTE¹⁴, O. SAHIN¹²⁴;
¹Biol. Sci., ²Physics, ³Pathology and Cell Biol., ⁴NeuroTechnology Ctr., ⁵Neurosci., Columbia Univ., New York, NY

Abstract: Synapses are mechanically interesting structures. They are enriched with dynamic actin networks and undergo fast twitching. Also, axons are under physiologically critical mechanical tension and a variety of trans-synaptic adhesion proteins tightly connects pre- and post-synaptic terminals. While biochemical, morphological and electrophysiological characteristics of synapses and neurons have been widely investigated, little is known about the mechanical behaviors of synapses and measuring their mechanical properties remains difficult. Here we develop an approach to study the mechanical behaviors of synapses using torsional
harmonic atomic force microscopy (TH-AFM). TH-AFM relies on a T-shaped cantilever and is capable of mapping mechanical properties and topographic features in living neurons with low indentation distance (<30 nm), low force (picoNewton scale) and high speed, suitable for delicate biological samples. We report that active mature synapses are substantially stiffer (up to 20 fold) than other neuronal structures (e.g. somas, dendrites) and the stiffness of synapses falls into a wide range. We combined optical microscopy with TH-AFM to record the morphology and stiffness of synapses in live rat hippocampal neuron cultures. To verify the identity of synapses, we performed post hoc immunofluorescence staining after AFM imaging and found that all stiff synapses are mature excitatory synapses. On the other hand, not all mature excitatory synapses reveal high stiffness under AFM. High magnification fluorescence images indicated that there are two subtypes of synapses: spiny and shaft. We hypothesize that the stiff synapses revealed by AFM are spiny synapses. To elucidate such heterogeneity, we are combining transmission electron microscopy with TH-AFM and aim to acquire the ultrastructure of stiff synapses and compare them with synapses that do not show high stiffness. Additionally, we monitored the activity of synapses with FM dyes, which bind to cell membrane and get internalized after presynaptic terminals release neurotransmitters, generating fluorescent puncta at active synapses. We showed stiff synapses are also active, suggesting stiffness may be related to synaptic activity. The extremely high stiffness could help maintain the unique morphology of synapses in the presence of strong adhesion forces and those mechanical processes may be important in synapse formation and function.


Poster

541. Novel Assays

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 541.09/CC50

Topic: G.04. Physiological Methods

Support: MEXT Regional innovation Strategy Support Program
       Nakatani Foundation

Title: Conductive polymer based silk electrode for cell activity measurement
Authors: *K. TORIMITSU, H. TAKAHASHI, Y. TAKIZAWA, S. WATANABE; Tohoku Univ., Sendai, Japan

Abstract: Usage of conductive polymer as a biocompatible soft electrode for neural activity measurement is one of our interest. We reported already a poly(3,4-ethylenedioxythiophene)-poly(styrenesulfonate) (PEDOT-PSS) improved the biocompatibility and the impedance of the electrode. Continuous monitoring of neural stem cell differentiation (from rat embryo striatum), neural cell development and network formation was achieved using this material. Here we report the formation of flexible electrodes using silk fiber for implantable electrode. Polymerization of the fiber with the conductive polymer allowed us for less-destructive and longer measurement. Brain neural activities or muscle activities of mouse and chick were measured with this electrode. Stimulation experiments with this electrode indicated stable contacts during measurements. Flexible characteristics of the electrode would be important for stable measurements. As the conductive polymer modified silk electrode is a flexible and biocompatible method, stable activity monitoring could be achieved for a primary evaluation of physiological cell conditions.


Poster

541. Novel Assays

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 541.10/CC51

Topic: G.04. Physiological Methods

Title: Micro-cautery based on photodeposition of zinc nanoparticle onto an optical fiber to prevent internal hematoma in abdominal and pelvic regions in adult rats

Authors: *C. F. PASTELIN, P. ZACA-MORÁN, G. F. PÉREZ-SÁNCHEZ, F. CHÁVEZ, C. MORÁN;
Univ. Autonoma de Puebla, Puebla, Mexico

Abstract: This study shows an experimental setup of a micro-cautery based on zinc nanoparticles photodeposited onto the core of an optical fiber (Figure 1). This micro-cautery represents an attainable option for the cauterization and coagulation processes that perform blood vessel hemostasis. The interaction between the laser radiation source and the metallic nanoparticles produces a precise micro-heat source, which is controlled by the power source’s
intensity. That source can reach temperatures up to 200 °C / 392 °F, which cover a diameter of ~10 µm. The aim of the experiment was the containment of the bleeding in a rat model with 3 to 4 months old coming from the CIIZ-V strain. The results show that the micro-heater obliterates smooth muscle from blood vessels and joins the tissue in approximately 3 seconds. At the same time, the coagulation processes is activated thermically, which makes the containment of hematoma possible. This is the first micrometric cautery based in the performance of nanoparticles.


Poster

541. Novel Assays

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 541.11/CC52

Topic: G.04. Physiological Methods

Title: Engineering a system to monitor home cage feeding behavior in rodents

Authors: *A. V. KRAVITZ, K. P. NGUYEN;
NIDDK, Natl. Inst. of Hlth., Bethesda, MD

Abstract: Studies have stressed the role of energy imbalance in the development of obesity, with food intake exceeding energy expenditure. To study mouse models of obesity, researchers need to accurately measure food intake; however, the most common method for monitoring food intake involves manual periodic weighing of food, which is time consuming, imprecise, labor intensive, and cannot measure feeding patterns. Commercial systems exist that can automatically capture high-resolution information about food intake, but can cost thousands of dollars for each cage, making it difficult to run high-throughput feeding experiments. Here, we engineered a feeding system that: (1) is low-cost, (2) is home cage compatible, and (3) can measure both food
intake and feeding patterns over multiple days. The device combines a custom-designed 3D printed housing with a microcontroller and off-the-shelf electronic parts to automatically deliver food tablets and record the frequency of food retrieval - all of this is achieved at a low battery consumption of approximately 0.15µAh. Pellet removal is sensed by a photointerrupter containing an infrared emitter with an opposing detector - when a tablet is sitting in the well, it interrupts the light signal, which then sends a low signal value to the system. The opposite occurs once a tablet is removed; this action then propagates the recording of a timestamp of food retrieval to an SD card and initiates the delivery of a replacement tablet into the well. Additionally, the design allows for interchangeable parts to accommodate food tablets of differing sizes such as 20 and 45mg. To validate the operation of the device, food retrieval was measured with the activation of agouti-related peptide neurons (AgRP) expressing in the arcuate nucleus (ARC) in mice, which have been implicated in driving food consumption and food-seeking behaviors. Data analyzed showed an overall increase in grams consumed, and more interestingly, demonstrated a marked increase in frequency of eating bouts of irregular patterns during the onset of photostimulation. Collectively, the dispenser offers a reliable method to accurately administer and record food retrieval for running low-cost, high-throughput feeding experiments.

Disclosures: A.V. Kravitz: None. K.P. Nguyen: None.
their lifespan to more than 36 hours. This system controls the temperature of the incubated artificial cerebral spinal fluid (aCSF) while continuously passing the fluid through a UVC filtration system and simultaneously monitoring temperature and pH. The combination of controlled temperature and UVC filtering maintains bacteria levels in the lag phase and leads to the dramatic extension of the brain slice lifespan. Brain slice viability was validated through electrophysiological recordings as well as live/dead cell assays. This system benefits researchers by monitoring incubation conditions and standardizing this artificial environment. It further provides viable tissue for two experimental days, reducing the time spent preparing brain slices and the number of animals required for research.

**Disclosures:** P.B. Breen: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); The Braincubator.

J.W. Morley: None. J. Tapson: None. A. van Schaik: None. Y. Buskila: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); The Braincubator.

**Poster**

**541. Novel Assays**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 541.13/CC54

**Topic:** G.04. Physiological Methods

**Support:** MOST-103-2321-B-182A-001

**Title:** Magnetic resonance imaging/diffusion tensor imaging of defunct baroreflex that underpins mortality in a rat model of hepatic encephalopathy

**Authors:** *C.-Y. TSAI, S. H. H. CHAN; Ctr. for Translational Res. in Biomed. Sci., Kaohsiung Chang Gung Mem. Hosp., Kaohsiung, Taiwan

**Abstract:** Hepatic encephalopathy (HE) is a common syndrome observed in patients with liver cirrhosis. In acute HE, it is a clinical emergency associated with 50-90% mortality in patients without liver transplantation. Since baroreflex is responsible for maintaining blood pressure (BP) and heart rate (HR), we reasoned that dysfunction of this regulatory mechanism underlies the high mortality associated with HE. In this study, we tested this hypothesis using a thioacetamide (TAA)-induced acute liver failure model of HE. In Sprague-Dawley rats, injection intraperitoneally of TAA (300, 400 or 500 mg/kg) at 24 h intervals for 3 consecutive days dose-
dependently increased the clinical grade of HE severity, mortality rate, and liver damage. 24-h BP and HR signals recorded by radiotelemetry in conjunction with on-line and real-time spectral analysis showed that HR was essentially maintained and cardiac vagal baroreflex was sustained until the abrupt occurrence of asystole that signifies cardiac death. On the other hand, drastic reduction in BP began to appear on Day 3, and at an accelerated rate on Day 4. More intriguingly, an index for baroreflex-mediated sympathetic vasomotor tone began to decrease on Day 2, and reached zero on Day 4, signifying clinically the occurrence of brain death. Tractographic analysis using magnetic resonance imaging/diffusion tensor imaging of the medulla oblongata revealed that the functional connectivity between the nucleus tractus solitarii (NTS) and nucleus ambiguus (NA), the origin of the vagal innervation of the heart, was sustained until shortly before asystole took place during experimental HE. On the other hand, the connectivity between the NTS and rostral ventrolateral medulla (RVLM), the origin of sympathetic innervation of blood vessels, was progressively disrupted, concurrent with impairment of baroreflex-mediated sympathetic vasomotor tone as detected by radiotelemetry. We conclude that sequential disruption of the connectivity between the NTS and NA and NTS and RVLM that underpins impairment of both arms of baroreflex accounts for the high mortality associated with HE.

Disclosures:  C. Tsai: None. S.H.H. Chan: None.

Poster

541. Novel Assays

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 541.14/CC55

Topic: G.04. Physiological Methods

Support: NIH Grant EY021624

Research to Prevent Blindness Unrestricted Grant

Title: Design of a neurovascular unit device using tissue engineering techniques for the study of cerebral microvascular permeability in stroke

Authors: *D. M. SANCHEZ-PALENCIA, M. SAINT-GENIEZ, J. ARBOLEDA-VELASQUEZ;
Schepens Eye Res. Inst., Boston, MA
Abstract: Our objective is to develop an *in vitro*, flow perfused, 3D biologic neurovascular unit (NVU) device, that stimulates the self-organization of vascular and perivascular cells into a sprouting capillary bed connecting with neural components for oxygenation. This model may be useful in the study of cerebrovascular permeability as relevant to stroke. We manufactured a 1 mm inner diameter (ID), 10 mm long, 75 um thick microvascular graft using dehydrated small intestinal submucosa (SIS) as a functional acellular extracellular matrix scaffold. SIS is a material widely used in tissue engineering applications and has been found to promote the *in vivo* regeneration of small and large arteries (4.5-8.0 mm ID). The micrograft was attached to a perfusion loop (3.0 ml/min flow rate, 5.4 dyn/sqcm shear stress) within an airtight chamber, and was cultured for 3 days with human umbilical vessel or retinal endothelial cells (HUVECs, HRECs respectively) seeded on the luminal surface and human or bovine retinal pericytes (HRPs, BRPs respectively) on the abluminal surface. Graft was imaged afterwards using immunofluorescence for lectin and smooth muscle actin (SMA). Monocultures with no perfusion showed that HUVECs, HRECs and HRPs adhered to SIS grafts and migrated inside the scaffold after seeding for 2h (HUVECs and HRPs). When placing the graft on top of confluent HRPs seeded on a culture dish, HRPs detached from the dish and attached to the graft after 6 days. Studies of the extent of cellular coverage and infiltration of the graft with HUVECs and BRPs in coculture and under flow perfusion for 3 days are ongoing. Further studies are forthcoming for assessing the ability of the neovessels to connect with neurospheres located in the vicinity. So far, our NVU device has promising results as a useful tool for modeling the increase of vascular permeability after cessation of flow in stroke.


Poster

541. Novel Assays
Title: Evaluation of the N-Methyl-D-Aspartate gated ion channel by an automated electrophysiology instrument designed for fast fluidic exchange

Authors: J. WEBBER, J. TANG, M. KASSINOS, B. ZOU, *P. MIU;
Drug Discovery, Mol. Devices, LLC, Sunnyvale, CA

Abstract: Automated electrophysiology instruments have become indispensable tools in enhancing throughput requirement for compound validation in drug screening. IonFlux is an automated electrophysiology instrument with a state-of-the-art microfluidic system that enables rapid solution exchange. In this study, we explored the capability of IonFlux solution exchange system to measure the N-Methyl-D-Aspartate (NMDA) receptor mediated currents. The NMDA gated ion channel is activated by glutamate, the primary excitatory neurotransmitter in the central nervous system (CNS). Functional impairment of the NMDA receptor causes a variety of CNS-related diseases thereby making it a prime therapeutic target for drug discovery. Upon glutamate binding, the agonist-bound NMDA receptor complex transitions from an activated (open) state to a desensitized state, which is characterized by a reduction in ion permeation through the transmembrane ion channel pore. Hence, precise measurement of maximum current flux through an open NMDA ion channel pore depends on the capability of a rapid solution exchange in order to mitigate the role of desensitization. Using HEK293 cells stably expressing NR1-NR2B subtype of the NMDA receptor, we characterized biophysical properties of NMDA ion channel in the presence and absence of extracellular magnesium. We compared data gathered on IonFlux with known NMDA receptor agonists, antagonists and positive allosteric modulators to those reported in literature using conventional patch clamp techniques. Our results showed that potency measurements are comparable to those obtained from conventional patch clamp. In conclusion, the microfluidic design of IonFlux has proven to be as effective as the piezo driven perfusion system, which is typically used in conventional patch clamp recordings for desensitizing ligand-gated ion channels.


Poster

541. Novel Assays

Location: Hall A
**Title:** Novel microfluidic blood-brain barrier neurovascular culture device

**Authors:** *J. A. BROWN*¹, D. MARKOV¹, V. PENSAEBE¹, V. ALLWARDT¹, D. NEELY², M. SHI¹, Q. YANG¹, O. HOILETT¹, P. SAMSON¹, L. J. MCCAWLE¹, D. WEBB¹, J. P. WIKSWO¹; ¹Vanderbilt Univ., Nashville, TN; ²Vanderbilt Univ. Med. Ctr., Nashville, TN

**Abstract:** The blood-brain barrier (BBB) acts as the gatekeeper between the central nervous system and the rest of the body. It is the responsibility of the BBB to facilitate the entry of required nutrients into the brain and exclude potentially harmful compounds; however, this critical and complex structure remains difficult to model in vitro. Accurate in vitro models are necessary for understanding how the BBB forms and functions and for evaluating drug penetration across the barrier. Many models fail to support all the cell types involved in BBB formation and/or lack the shear forces created by flow needed for mature tight junction formation. To address these issues and establish a more faithful in vitro BBB model, we have designed and fabricated a microfluidic neurovascular unit (NVU) that comprises a vascular chamber and a brain chamber separated by a porous membrane that allows cell-cell communication between endothelial cells, astrocytes, and pericytes. The NVU permits independent perfusion of both sides of the membrane and allows for the delivery of high shear flow on the luminal endothelial surface while simultaneously providing low flow to co-cultured cells within the brain chamber. The integrity of the blood-brain-barrier within the NVU has been validated with both FITC-dextran diffusion and transendothelial electrical resistance. The NVU has enabled in vitro modeling of the BBB using all human cell types and sampling of effluent from either side of the barrier.

**Poster**

**541. Novel Assays**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 541.17/CC58

**Topic:** G.04. Physiological Methods

**Support:** UK SMART Rounds, Technology Strategy Board, UK Government Grant REF: 710671

**Title:** Muscle stimulation for haptic feedback in immersive environments

**Authors:** *D. Y. BUCKLEY¹, Y. CHAI¹, A. SERENA¹, C. PIANCASTELLI¹, Y.-C. CHOU¹, C. BIANCHINI¹, M. MARCHWICKI¹, A. VENDITTI¹, L. FENES¹, J. GRAUBINS¹, G. BOISSELET¹, P. S. BLOOMFIELD²;¹UNIT9 Ltd., London, United Kingdom; ²Inst. of Clin. Sci., Imperial Col. London, London, United Kingdom

**Abstract:** Interfaces between users and technology are developing rapidly in the gaming industry. Haptic feedback in controllers and joysticks has provided users with a level of immersion for consoles, however feedback remains an artificial sensation, relatively far removed from the appropriate physiological context. The development of Oculus rift virtual reality (VR) for consumers has created opportunities for more immersive environments. A large disconnect from the VR setup relates to the lack of environmental feedback. Here we present the latest from our Pretender Project (working title), where haptic feedback utilising transcutaneous electrical nerve stimulation (TENS) is being developed to provide sensation and muscle movement control to be applied to a VR environment. Using a TENS Arduino setup with electro-myographic (EMG) style electrodes we demonstrate how utilizing computer programmed muscle group stimulation, we are able to control limb movements with temporal and spatial accuracy. The experimental setup we have developed operates in a safe range of current (0-80 mA), voltage (0-40 V, 500 ohm) and frequency (up to 250 Hz). The current project aim is to develop the technology for muscle movement with full spatial control, however TENS stimulation can be used for sensory feedback as well as control of movement, which is a future advance to be made. The present investigation demonstrates the muscle groups we can functionally manipulate to provide accurate stereotyped movements in this haptic feedback paradigm. While this technology
is currently being developed primarily for haptic feedback and a gaming environment, there is scope for it to be applied to rehabilitative medicine and can be combined with other technology and research platforms for experimental application.


**Poster**

**541. Novel Assays**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 541.18/CC59

**Topic:** G.04. Physiological Methods

**Title:** Information content of video tracking and pressure-sensor derived signals for the discrimination of mouse behaviour in health versus disease

**Authors:** M. CARRENO MUNOZ¹, K. LÓPEZ DE IPIÑA², S. PIETROPAOLO¹, A. MOUJAHID², A. FRICK¹, *X. LEINEKUGEL¹;

¹Neurocentre Magendie, INSERM U862, Bordeaux, France; ²Univ. del País Vasco, San Sebastian, Spain; ³CNRS, UMR 5287, Talence, France

**Abstract:** Behavioural phenotyping is a required step to exploit the multitude of transgenic mouse models and potentially useful pharmacological agents made available by academic and industrial pharmaceutical research. It is based on the detection of animal movement. Pressure sensors using the piezoelectric technology can provide extremely detailed and precise information regarding animal movement, that can be a nice complement to video signal to analyze laboratory animal behaviour in a variety of protocols, including freely moving in an open field. One interesting thing about such movement-related signal is that it reflects the summed activity of all the muscles of the animal. Depending on the coordination of these myriads of muscles, their mechanical piezo signature can sum up or cancel each other. This is therefore a very rich but also very complex signal, that can be used to characterize the behavioural phenotype of the animals under study. However, behavioural phenotyping is a very complex issue and the more precise and sensitive tool, the more potential categories to classify behaviours, so that it becomes a very tedious and difficult task. Accordingly, it has been reported recently that trained human operators requested to attribute each second of time to a specific behaviour have major problems classifying up to 44% of individual seconds to any specific behaviour. This has lead our interest towards synthetic parameters such as Permutation Entropy,
which can grasp biological system's complexity in a quantified manner. Permutation Entropy measurement can be performed automatically without human supervision and is cost effective. It has already been used with some success to detect the effects of drugs on rat locomotor behaviour or to detect differences between the behaviour of fishes exposed to pollution. We have tested the use of Permutation Entropy applied to spatial tracking and piezo signal, and report that it could discriminate between wild type and transgenic mice models of pathology affecting behaviour in an open field.

Disclosures: M. Carreno Munoz: None. K. López de Ipiña: None. S. Pietropaolo: None. A. Moujahid: None. A. Frick: None. X. Leinekugel: None.

Poster

542. Data Analysis

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 542.01/CC60

Topic: G.07. Data Analysis and Statistics

Title: How to visit 0.5% of 15,000 possible posters? Automated poster visit scheduler for SfN

Authors: *D. ACUNA*¹, T. ACHAKULVISUT², K. KORDING³;
¹Rehabil. Inst. of Chicago, Chicago, IL; ²Biomed. Engin., ³Northwestern Univ., Chicago, IL

Abstract: SfN is one of the largest scientific conferences in the world, with around 15,000 posters presented over 4 days. Any visitor faces a hard question: which poster should I visit? Here we apply a large-scale matching between visitors and posters that produces an automated scheduler for each of the visitors. Our solution assigns no more than 50 visitors per poster and schedules around 20 posters per day per visitor. The program also considers that visitor should not visit posters of his or her own lab. Importantly, our algorithm only uses the abstracts of the posters being presented, requiring little human intervention. Our work would be better suited for a presentation as dynamic poster, where we will display the web-based tool and let fellow scientist try it live. There will be two ways to obtain a schedule. If a visitor is the co-author of a poster, the schedule will be precomputed. Otherwise, the visitor can select posters and the system will suggest a schedule with posters that are similar to those chosen. We built a proof of concept based on SfN 2014’s abstracts: http://sfn.scienceofscience.org Being SfN one of the largest conferences in the world, deciding which poster to visit is a time consuming problem that can be readily solved using machine learning. Our future goals are to extend this work to other massive conferences and also specialize it to other neuroscience conferences such as COSYNE 2016.
Multidimensional imaging of brain slices or cell cultures: acquisition and analysis

Authors: *N. N. KARPUK, T. KIELIAN;
Pathology & Microbiology, Univ. Nebraska Med., Omaha, NE

Abstract: Brain slice imaging is successfully used in numerous studies. However, changes in cellular integrity and tissue structure during brain slice preparation can augment autofluorescence and non-specific staining. In addition, fluctuations in dye photo-bleaching and fluorescence intensities in living tissues create challenges to obtain reliable imaging data on brain slices using conventional single-spot acquisition methods. This issue is further confounded by difficulties arising when comparing data between different slice preparations. Here we describe an automated method that simplifies image acquisition in multiple locations from one or more brain slices using a programmable 3-dimensional motorized stage (3DMS-285, Sutter Instruments) synchronized with AxioVision software (Zeiss, Germany) through a parallel PC port and tracked in real-time by video monitoring using a low resolution objective (2.5x) and image panorama settings. For image acquisition, we have developed software integrating Visual Basic of AxioVision and a modified demo version for the MP-285 controller (Sutter Instruments). By imaging multiple locations, this design effectively reduces light exposure in any one area that together minimizes dye photo-bleaching, improves statistical values, and improves data reliability.
Program#/Poster#: 542.03/CC62

Topic: G.07. Data Analysis and Statistics

Support: NIH Big Data to Knowledge (BD2K) Initiative under U54EB020403
ADNI NIH Grant U01 AG024904

Title: Test-retest reliability of cortical parcellations in 165 healthy adults for multi-site analyses in the ENIGMA consortium

Authors: *J. FASKOWITZ, D. P. HIBAR, P. M. THOMPSON, N. JAHANSHAD;
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Abstract: ENIGMA, and other large neuroimaging consortia, pool results from different imaging studies to maximize power to detect subtle, yet common, effects on brain structure including those from single DNA variants. A challenge of multi-site collaboration is harmonizing imaging measures derived from different MRI scanning protocols. FreeSurfer software is widely used for the automatic parcellation of various cortical regions of interest (ROIs). Given the key role of the human cortex in cognition and disease, discovering factors that shape the cortex is critical. Here, 165 healthy elderly participants from the ADNI dataset (49.1% F; mean age: 74.0 +/- 6.3) were scanned across 45 sites within North America with T1-weighted MRI (3.0 tesla; (1.2 mm)^3 voxel) at two time points 3 months apart. We assessed test-retest reliability within and across 4 releases of FreeSurfer (5.3, 5.1, 5.0, and 4.5) on a Unix HPC-cluster. Each version of the recon-all pipeline was run with only one non-default set of parameters (--proto-iter 1000; --distance 50) to optimize the procedure for 3T scans, as suggested by developers. ENIGMA protocols were used to perform quality control of the outputs to find visual and statistical outliers, and then to obtain measures for 68 ROIs. Test-retest reliability was calculated using the intra-class correlation coefficient (ICC) for each ROI and across numerous versions of FreeSurfer. ICC for ROIs within a version was generally high. ICC specifically for the medial orbito-frontal, temporal pole, and entorhinal region was lower (ICC<0.80), possibly due to common image artifacts in these areas. Between versions, ICC was high, but comparisons to v4.5 were least consistent. This line of research has the potential to aid the neuroimaging community to make informed decisions when assessing the reliability of meta-analyzed results across studies.

Poster

542. Data Analysis

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 542.04/CC63

Topic: G.07. Data Analysis and Statistics

Title: A MATLAB toolbox to tame the torrent: efficient video processing routines for wide-field Ca\textsuperscript{2+} fluorescence imaging in awake behaving animals

Authors: *M. BUCKLIN\textsuperscript{1}, H.-A. TSENG\textsuperscript{2}, A. I. A. MOHAMMED\textsuperscript{2}, X. HAN\textsuperscript{2}; \textsuperscript{1}Biomed. Engin., Boston Univ., Chelsea, MA; \textsuperscript{2}Boston Univ., Boston, MA

Abstract: The latest generation of genetically encoded calcium sensors deliver a substantial boost in signal strength. This - combined with equally critical advances in the semiconductor
technology available in scientific cameras - enables high-throughput detection of neural activity in behaving animals using traditional wide-field fluorescence microscopy. We are now able to record continuous activity of greater than one thousand neurons in a behaving mouse for hours at a time over several weeks. This breakthrough in signal strength is manifested by a considerable increase in the spatial and temporal resolution we may use to detect and record neural activity, which will undoubtedly strengthen our competence to conduct brain research. However, the tremendous concomitant increase in data flow forces new challenges in downstream image processing and data analysis, and prompts a reexamination of traditional routines used to process data in neuroscience. We developed an open-source MATLAB toolbox for efficiently analyzing and visualizing large imaging data sets. The toolbox is capable of interactive or fully automated use. The widespread usage of MATLAB in neuroscience communities lends potential for greater usability and easier adaptation to software developed in this environment. While easier development has traditionally presumed crippling sacrifices to computational performance, we've constructed an assembly of efficient routines - exploiting updated toolboxes for multi-core and GPU parallel computing among other advancements - that enables processing and distillation of massive experimental stores of data into rich yet usable form in a practical timely manner. This software package makes provides a library of image pre-processing routines such as for motion-correction and contrast enhancement, optimized for batch-processing of dynamic fluorescence video, and additionally automates a fast unsupervised ROI detection routine, a necessary feature for imaging data-sets where the numbers of identifiable single cells reach into the thousands. This is achieved through various spatial morphological enhancements mixed with probabilistic functions that consider the local spatial and temporal characteristics from an image sequence to generate, correlate, combine and refine distinguishable regions of cellular activity.

Disclosures:  M. Bucklin: None.  H. Tseng: None.  A.I.A. Mohammed: None.  X. Han: None.

Poster

542. Data Analysis

Location: Hall A

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Program#/Poster#: 542.05/CC64

Topic: G.07. Data Analysis and Statistics

Support: BGRO, Georgetown University

Title: Automated control of associative learning and spatial decision making in freely swimming zebrafish, danio rerio
Abstract: Associative conditioning is an important neural mechanism that allows animals to adapt their behavior to environmental demands. Current methods used for associative conditioning often involve human intervention, which is labor-intensive, stressful to animals, and can introduce noise in the data. We have developed a relatively simple yet flexible paradigm for training zebrafish, Danio rerio, and possibly other experimental animals, that requires minimal human intervention. Our methodology combines stimulus presentation through LED’s and/or an underwater output transducer with video tracking of fish (e.g. using iDTracker, Pérez-Escudero et. al., 2014). Food reward is delivered through a microprocessor (Arduino) controlling a small robotic arm and feeder via stepper motors. This allows full automation of reward-based place preference conditioning of adult zebrafish to visual and auditory stimuli. “Ardulink”, a JAVA facility, allows simultaneous implementation of communication protocols with Arduino via a user-friendly interface (Version 0.4.2; Zu, 2013). Our software’s user-definable settings enable either classical or operant conditioning via customized multi-day scheduling of training parameters, as well as precise control of the timing, location and intensity of stimulus presentation. During operant conditioning, zebrafish trigger motion sensors to obtain a small reward. The standardized training and tracking procedure facilitates comparison of results across multiple training runs within a multi-day trial. We have been successful in training zebrafish to discriminate between visual cues (different colored LEDs) over a 3-day session with 6 training runs per day. Discrimination of auditory cues can take 4 or more days. Our method provides a quick and efficient way to exhaustively test sensory capabilities of freely swimming zebrafish and to screen drugs as well as test effects of CRISPR-based and optogenetic modification of neural circuits on learning and memory under naturalistic conditions.


Poster

542. Data Analysis

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 542.06/CC65

Topic: G.07. Data Analysis and Statistics
Support: NIH Grant R01HD078561
NIH Grant R21HD069001
NIH Grant R03NS091587

Title: Optimization of ex vivo high-resolution mouse diffusion tractography at the gray/white matter border

Authors: P. KABARIA1,3, *G. DAI4, E. TAKAHASHI2;
1Div. of Newborn Medicine, Dept. of Med., 2Boston Children's Hosp., Boston, MA; 3Dept. of Behavioral Neurosci., Northeastern Univ., Boston, MA; 4Radiology, Martinos Center/Mgh, Charlestown, MA

Abstract: One commonly used method for creating three dimensional brain fiber trajectories is diffusion tractography. A major challenge with using this method is to identify accurate, continuous fiber pathways through the gray/white matter where axons turn in close to 90 degrees. This study seeks to determine optimal sets fiber reconstruction parameters in order to increase the accuracy of diffusion tractography. The sets of parameters examined consist of combinations of six algorithms (DTI-FACT, DTI-2nd-order Runge Kutta, DTI-Interpolated Streamline, DTI-Tensorline, HARDI-FACT, HARDI-2nd-order Runge Kutta), nine weight masks options (No Weight Mask, 1.25, 1.50, 1.75, 2.00, 2.25, 2.50, 2.75, 3.00), and seven angle thresholds (20, 30, 40, 50, 60, 70, 80 degrees). These parameters were examined over ten adult mice with two different pathways: motor and callosal. The accuracy of the images with each set of parameters was compared to the “gold standard” set by tracer studies in the Allen's Brain Atlas for the motor pathway, and to the well-known description of corpus callosum tracts for the callosal pathway. Each set of parameters were scored using a scoring method that took into account the amount of accurate tracts and the amount of spurious tracts. An average score was determined for each set of parameters for each pathway, resulting in a compilation of average scores for every combination of algorithm, weight mask, and angle threshold for each pathway. For the motor pathway, DTI-FACT and DTI-Interpolated Streamline have the overall highest average scores compared to the other algorithms (scores of 1.74 and 1.69, respectively) for all of the weight mask variables. Weight Mask 2.50 has the overall highest average scores for four of the six algorithms, more than the other weight masks. The angle threshold of 20 degrees has the highest average scores for three of the six algorithms, more than the other two angle thresholds. For the callosal pathway, DTI-FACT and DTI-2nd-order Runge Kutta have the overall highest average score (2.9 for both), for all of the weight mask variables. Weight mask 3.0 has the overall highest average scores for three of the six algorithms, more than the other weight masks. The angle threshold of 20 degrees has the highest average scores for four of the six algorithms, more than the other two angle thresholds. From these findings, the optimal sets of parameters are combinations of DTI-FACT, DTI-Interpolated Streamline, or DTI-2nd-order Runge Kutta, with weight masks of 2.50 or larger, and angle thresholds of 50 degrees or smaller. It can be
concluded that using sets of parameters involving these range of variables will result in more accurate images.

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**Poster**

**542. Data Analysis**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 542.07/CC66

**Topic:** G.07. Data Analysis and Statistics

**Support:** ANR-14-CE13-0003

**Title:** Spying circus: a new software for fast, scalable spike sorting of large-scale extracellular recordings

**Authors:** *P. YGER¹, O. MARRE²;
¹Inst. De La Vision, Paris, France; ²Inst. de la Vision, Paris, France

**Abstract:** Understanding how assemblies of neurons encode information requires to record large populations of cells in the brain. In recent years, large multi-electrode arrays and silicon probes have been developed to record simultaneously from hundreds of electrodes packed with high density. However, these new devices challenge the classical way to do spike sorting in several ways. First, the large number of electrodes preclude approaches based on manual clustering, and even automatic approaches need to be fast enough to handle the growing amount of extracellular data. Second, the density of the electrodes is now high enough so that a single spike is often detected on many electrodes, so that the different channels must be processed simultaneously. Third, within those large and dense array of electrodes, overlapping spikes are becoming the rule rather than the exception, perturbing the classical clustering methods that cannot easily capture the synchronous occurrences of spikes from different cells. Here we developed a new software solving these issues and allowing fast and scalable spike sorting of large-scale recordings. To do so, we developed a highly automated algorithm composed of two main steps: 1) a "template-finding" phase to extract putative cell’s templates, i.e. patterns of activity evoked over many electrodes when neurons fire an action potential; 2) a "fitting" phase where the templates are matched onto the raw data to resolve the location of the spikes. For the template-finding phase, performed only once on a subset of the data, we start by detecting all the possible times in the raw data that could contain a spike. Spikes are then clustered into groups using state of the art density-based clustering [1]. We then extract the template corresponding to each group, and in
the fitting phase, we match those templates onto the raw data with a matching-pursuit method allowing amplitude variation for each template, inspired from [2]. The algorithm is written in Python and is entirely parallelized on CPU and GPU to handle large amount of data, almost in real-time. Moreover, a graphical user interface is provided to check the output of the algorithm and refine the results of the sorting. We are currently testing our algorithm with large-scale data from in vitro and in vivo recordings, estimating its performance on data with “ground truth”, i.e. cases where the solution to the sorting problem is at least partially known. References [1] A. Rodriguez and A. Laio, Clustering by fast search and find of density peaks Science, 344(6191):1492-1496 [2] O. Marre et al, Mapping a Complete Neural Population in the Retina, Journal of Neuroscience 32(43): 14859-14873

Disclosures: P. Yger: None. O. Marre: None.

Poster

542. Data Analysis

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Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 542.08/CC67

Topic: G.07. Data Analysis and Statistics

Title: Spatial features of reliably constructed structural brain networks

Authors: M. Y. MAHAN\textsuperscript{1}, *A. P. GEORGOPOULOS\textsuperscript{2};
\textsuperscript{1}BICB, \textsuperscript{2}Neurosci, Univ. Minnesota, Minneapolis, MN

Abstract: Structural connectivity analyses rely on various methods to build graphs representing the anatomical brain. Reliable construction of these graphs is central to identifying valid network assessments across different study populations. Typically, reproducibility studies rely on multiple subject sessions and compare network measures across these sessions to categorize the validity of network metrics. However, many existing datasets contain only one session for each subject. Therefore, a fresh approach to constructing graphs with reliable network metrics requiring one session per subject would be valuable. For that purpose, we present a permutation-based approach to identify which structural graphs are reliable. Using the reliable graph, we apply novel spatial graph algorithms for discovering structural network patterns with age. To construct structural graphs, sMRI and DTI data were collected from 65 cognitively healthy women (32-74 years old). Four node definitions were used: (1) automated anatomical labeling (AAL), random construction with the number of nodes (2) \( \cong \) AAL, (3) \( > \) AAL, and (4) \( < \) AAL. Then, for each node definition, two edge definitions were used: streamline and probabilistic white matter tractography. For each node-edge construction method, both thresholded binary
matrices and nonthresholded weighted matrices were used. With each graph-construction type, 100 random permutations of the data were performed and graphs were constructed, resulting in 101 matrices for each graph-construction type. Characteristic network metrics were calculated for all graphs. To determine which graph-construction types are reliable, distances between subject and permuted matrices for each graph-construction type were calculated. The distances were evaluated for each graph-construction type, whereby longer distances indicate higher reliability. Based on these results, the most reliable graph-construction type was retained for further analysis. The final analysis focused on constructing an inclusive structural network. To accomplish this, additional metrics were added on the nodes, namely, three labels: gray matter thickness, volume, and surface area. For this cohesive structural graph, novel and characteristic spatial graph metrics were calculated from each brain area, lobe, hemisphere, and whole brain, resulting in a structural network assessment for each subject. The effect of age was measured by performing a multivariate regression. Through an iterative, stepwise procedure those metrics that varied systematically with age were retained and used to construct an overall assessment of how structural network patterns changing with age.

Disclosures:  M.Y. Mahan: None.  A.P. Georgopoulos: None.

Poster

542. Data Analysis

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Topic: G.07. Data Analysis and Statistics

Support: NSF Grant BCS1129855

Title: A probabilistic latent factor approach for multi-subject fMRI data modeling

Authors: *P.-H. CHEN, P. J. RAMADGE;
Princeton Univ., Princeton, NJ

Abstract: A critical aspect of multi-subject fMRI analysis is the aggregation of data across multiple subjects, each of whom has a distinct functional topography. Recently, considerable progress has been made on this problem using both anatomically local functional alignment [Sabuncu 2009, Conroy 2009, Conroy 2013], and subject specific rotations of the data within anatomically selected regions of interest [Haxby 2011, Lorbert 2012]. We examine a factor analysis model for determining a small set of features that are shared within a local anatomical region of interest (ROI), or searchlight, across a group of subjects. If this ROI contains n voxels,
We seek to reduce the dimensionality of the fMRI data in this ROI from $n$ time series per subject, to $k$ brain maps (each containing $n$ voxels), for each subject, and a shared set of $k$ time series. We study a generative model to accomplish the stated across-subject factor analysis. We then formulate a probabilistic extension of this generative model. This leads to a constrained expectation-maximization (EM) algorithm for associated maximum likelihood estimation. Compared with previous approaches, e.g., [Haxby 2011], our formulation has the benefit of improved regularization of the estimate of the shared elicited response across subjects and better generalization to new data. More generally, a probabilistic formulation forms the basis for Bayesian treatments, enabling the natural incorporation of neuroscience domain knowledge into alignment modeling. Since it is a latent variable model, it can also be used as a building block for more complex models. We assess the effectiveness of the proposed method using fMRI datasets with distinct characteristics, and a various anatomical ROIs varying from ventral temporal cortex to the posterior medial cortex. In a first experiment, we use one half of the fMRI response to a movie viewing to learn the shared factors. Then using a held out subject, we pick a segment from the unused half of the movie data, and attempt to locate the matching segment using other subjects data with one-nearest neighbor classification based on the correlations between response segments. In a second experiment, we use movie viewing fMRI responses to learn the shared factor representation. Then for a held out subject, we conduct stimulus prediction on image viewing responses with support vector machine classifier with other subjects data as training set. In these experiments, we observe 5% to 18% average improvement in generalization accuracy over related methods including anatomical and functional alignment as well as several factor models.

Disclosures: P. Chen: None. P.J. Ramadge: None.

Poster

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Topic: G.07. Data Analysis and Statistics

Support: NIH Grant ZIA MH002783

Title: Multi-echo fMRI enhances reliability of brain-wide BOLD responses to a naturalistic movie
Abstract: As fMRI researchers increasingly tackle naturalistic, complex experimental paradigms, response reliability is often used in place of response amplitudes as a metric of each voxel’s responsiveness to a stimulus. A voxel’s response from one run is used as a model for the same voxel’s response in the other runs, a process that is repeated for each possible pairing of runs. Voxels with "reliable" responses are those that consistently show positive correlations across pairings (Hasson et al., 2004, Science). This approach is especially useful in cases where the mapping from stimulus to response is not well characterized, such as the free viewing of a naturalistic movie. Such studies have revealed many areas of consistent activation in both across-subject and within-subject groupings, inspiring more researchers to adopt the approach. But the relatively unconstrained nature of these paradigms translates to a low contrast-to-noise ratio, so response reliability measures have much to gain from de-noising techniques. To this end, we apply multi-echo independent component analysis (MEICA), a recently developed technique that has shown great promise in resting-state and task-based analyses, to data from the free viewing of a naturalistic movie. The MEICA technique uses a pulse sequence that collects signals from three echoes per TR, and then uses the variation of signals across echoes to distinguish BOLD-like signals from non-BOLD-like noise (Kundu et al., 2012, NeuroImage). In the current study, two subjects watched a 7-minute cartoon movie 16 and 17 times respectively across multiple days. We then analyzed the intra-subject correlations (intra-SC) of voxels across runs. The response reliability was assessed in this way both with and without MEICA de-noising. The MEICA results demonstrate that the movie evokes reliable brain-wide activation that is much more extensive than standard data collection and processing methods might suggest. When MEICA was used instead of standard single-echo processing, the number of voxels showing significant activation (FDR corrected q<0.05) increased from 21% to 47% in subject 1 and from 22% to 34% in subject 2. The voxels whose response reliability was revealed by MEICA but not by single-echo processing are significantly more likely to be in gray matter than in-brain voxels chosen at random (one-tailed binomial test, p<1e-15), suggesting that they are driven by neural activity. Preliminary analyses indicate that some of these areas are driven by high-level properties of the movie such as the presence of faces, emotional content, and motion.

Title: Probabilistic maps identify spatially variable features of large-scale resting-state functional connectivity brain systems

Authors: *E. M. GORDON*, T. O. LAUMANN*, B. ADEYEMO*, S. E. PETERSEN*;


Abstract: The human cortex is organized into large-scale, spatially distributed systems that can be described *in vivo* using a functional magnetic resonance imaging (fMRI)-based technique known as resting state functional connectivity (RSFC). The topologies of these systems have been described in group average data (Power et al., 2011). However, our recent work in single subjects suggests that individuals’ brain systems may have complex topological features that cannot be observed in group average data (Laumann et al., under review). This effect is likely because cross-subject averaging blurs small, spatially variable system features. Here, we use a novel approach to identify individual-level system features that show some consistency across a group of 120 healthy adults. We applied a template matching procedure to delineate brain systems in each individual. We then matched the discrete, discontiguous patches of these systems between each pair of subjects based on the geodesic distance along the cortical surface between two patches. Finally, we applied a community detection procedure (Infomap) to identify sets of system patches likely to represent the same cortical object across subjects. These sets were visualized as probabilistic maps of matched system patches. We observed that all system patches present in the group average systems were also present in more than 95% of subjects, and that the probabilistic maps of these patches tended to have very dense spatial distributions, with little variability in their location on the cortical surface. Notably, we also observed a number (~60) of system patches that were not observed in the group average systems. These patches were identified across many subjects (30% - 95%), but were relatively small (~250mm² median size across subjects). They also tended to have diffuse spatial distributions, indicating a large amount of spatial variability. Despite their small size and variable position, many of these
patches 1) were homotopically paired across cortical hemispheres; 2) replicated in a separate dataset of 108 healthy adults acquired on a different scanner; and 3) replicated using a different set of template systems. These findings increase confidence that the patches described here represent “true” system features in individuals’ brains. The identification of many small, spatially-variable features of large-scale brain systems suggests that the systems-level organization of the human brain is more complex than may have been previously appreciated. Further, the spatial variability of features described here may confound studies that assume equivalent brain systems are present across individuals in a cortical region.


Poster

542. Data Analysis

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 542.12/CC71

Topic: G.07. Data Analysis and Statistics

Title: Biomarkers of Neurodevelopmental disorders in Early Childhood: pilot study using functional near infrared spectroscopy

Authors: *A. A. ANDERSON¹, E. SMITH², V. CHERNOMORDIK¹, N. KARAMZADEH¹, F. CHOWDHRY¹, A. THURM², A. GANDJBAKHCHE³;
¹NICHD, ²NIMH, NIH, Bethesda, MD

Abstract: To understand the development of brain function there is a crucial need to quantitatively assess brain activation in early childhood, specifically for early intervention of neurodevelopmental disorders. The current brain imaging modalities (such as fMRI or PET), however, make it challenging to study the brain function at a young age, mostly due to patient movement or invasive nature of the study. Functional near infrared spectroscopy (fNIRS) is an emerging non-invasive brain imaging technology that is affordable, compact, and less susceptible to patient movement. Therefore, it becomes suitable for imaging the cortical activation in young cohorts. In this study, we used an fNIRS to assess functional biomarkers, oxy- and deoxy-hemoglobin, based on activation in prefrontal cortex (PFC) using specific tasks. We conducted two studies to compare the functional development of the brain: 1) in typical children from ages of 4-8 performing a Go/No-Go task and 2) in a group of 3-year-old typical and language delay (LD) toddlers watching a video. We introduced a novel parameter, Oxygenation Variability Index (OV index), directly obtained from fNIRS data. This index
measures the changes in oxygen saturation in PFC in frequencies related to cerebral autoregulation (<0.1 Hz). In a group of seventeen typical children, our results indicate that the OV index increased significantly with age between 4 and 6 years and decreased afterward, reaching a plateau. In 3-year-old toddlers, we noticed a significant difference in OV Index between LD (N=4) and typical toddlers (N=5), with the latter showing a higher level of OV Index in left PFC. Moreover, compared to the typical group, LD toddlers exhibited significant unilateral activation in PFC. These findings for the first time provide preliminary evidence to describe the relationship between the OV index and age in children, and differentiate the brain function at the early stage of neurodevelopmental disorders using fNIRS methodology.

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Poster

542. Data Analysis

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Topic: G.07. Data Analysis and Statistics

Support: Cumming Research Foundation

Title: Structure and function of the sources of thalami-cortical dysrhythmia in human, revealed by magnetic encephalography

Authors: *R. R. LLINAS*¹, M. N. USTININ¹², S. RYKUNOV¹, A. I. BOYKO², K. D. WALTON¹, G. RABELLO¹;
³Russian Acad. of Sci., Inst. of Mathematical Problems of Biol., Pushchino, Moscow Region, Russian Federation

Abstract: Magnetoencephalographic (MEG) results from patients suffering from thalamo-cortical dysrhythmia syndrome were studied using a novel data analysis methodology. Spontaneous brain activity data sets from xx patients were obtained using a 275-channel gradiometer VSM Medtech, at the Center for Neuromagnetism at Bellevue Hospital in of New York University School of Medicine. The NYU Institutional Review Board and Bellevue Hospital Research Protocol Review Group approved the study and an informed written consent was obtained from all subjects prior to the recording event. Detailed multichannel Fourier
spectrum was calculated from activity collected during 240 sec recording time sets, resulting in high frequency resolution (0.0024 Hz). Patients with thalamocortical dysrhythmia, are characterized by their abnormally high amplitude at Theta-band frequency. Such activity, observed in all patients, was then selected for computational further analysis. These multichannel spectrum recordings, were then decomposed to sets of functionally invariant entities, each one characterized by distinct frequency, amplitude and phase. Frequency-pattern analysis of these recording sets made it possible to find the spatial distribution of the MEG energy sources, and to estimate the activity dominant direction for each point in space. The next step of analysis included the generation of individual magneto-resonance images which made it possible to extract partial spectrum relating to thalamic spontaneous activity. The characteristic time series of this activity were then reconstructed from partial spectrum. The excellent localization results obtained, in fact, better than two cubic millimeters, allow us to conclude, that magnetic encephalography, combined with the novel methodology concerning precise frequency-pattern analysis, effectively reveals localization of the spontaneous activity of the deep brain sources. This novel method, allowing the reconstruction, via cooperative analysis, of time series from distinct brain localities, will provide new clues concerning both normal and abnormal human brain function.


Poster

542. Data Analysis

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 542.14/CC73

Topic: G.07. Data Analysis and Statistics

Title: Using multi-echo fMRI to increase task-based contrast-to-noise and response stability

Authors: *B. GUTIERREZ1, D. HANDWERKER2, J. GONZALEZ-CASTILLO2, V. ROOPCHANSINGH3, L. BUCHANAN2, P. BANDETTINI12;
1Section on Functional Imaging Methods, 2Functional MRI Facility, 3NIH/NIMH, Bethesda, MD

Abstract: Multi-echo fMRI denoising is based on the idea that the magnitude of a BOLD-weighted signal will increase with echo time (Posse 1999). Multi-echo independent component analysis (ME-ICA) takes advantage of this by looking to see if a signal’s strength increases across echoes implying a BOLD-like character. ME-ICA splits the data into ICA components, removes the components unlikely to represent BOLD fluctuations, and then reconstructs a
denoised time series with the remaining components (Kundu 2012, 2013). ME-ICA has been shown to effectively remove artifacts in resting-state fMRI, improving connectivity maps (Kundu 2013, Evans 2015). Here, we show how multi-echo processing techniques affect the results and stability of a single run block design task. Using the same task as Gonzalez-Castillo et al. 2012, we collected 103 and 104 5:28 minute block design runs for two subjects respectively across 9 days each. Statistical results were calculated on the middle echo (Echo2) time series (TE=29.7ms), an optimally combined (a TE weighted average of the 3 echoes) time series, and a ME-ICA denoised time series. Using two anatomically defined regions of interest, the Calcarine Sulcus (CS) and the Lateral Genticulate Nucleus (LGN), we investigate stability, reliability, and signal quality across runs in our single-echo and multi-echo time series. We fit a General Linear model (GLM) using a finite impulse response model to each run in each processing method. This allowed us to look at the hemodynamic response function (HRF) variability and calculate the contrast-to-noise ratio (CNR: magnitude of HRF/stdev of residual). For a single run in the CS, we observe a 0-20% increase in CNR from Echo2 to Optimally Combined and a 0-40% CNR increase from Echo2 to ME-ICA Denoised respectively. We also see CNR improvement from Optimally Combined to ME-ICA Denoised on average. In the LGN, a region showing significance in approximately 50% of runs (FDR corrected q < 0.05) and lower CNR in general, we see that over half of the time we get increases in CNR from Echo2 to Optimally Combined and from Echo2 to ME-ICA Denoised, but no visible improvement from Optimally Combined to ME-ICA Denoised. These results suggest reliable improvements in the CNR of single runs by using Multi-echo imaging and processing techniques. The variability in the amount of improvement across runs points to the potential to improve the multi-echo denoising algorithm to increase the consistency of the improvements.

Abstract: In previous work, we developed a model called Hierarchical Topographic Factor Analysis (HTFA) for efficiently discovering, representing, and computing with full-brain functional connectivity patterns in large multi-subject fMRI datasets. HTFA works by representing full-brain functional connectivity patterns using a set of spherical network “hubs” placed throughout the brain. Given an fMRI dataset, we can use Bayesian inference to compute the most probable number of hubs, the hub locations and sizes, and their activations over time (which are in turn used to infer hub-to-hub connectivity patterns). The result is a highly compact (low dimensional) representation of the full-brain connectivity matrix that is more efficient to compute with than voxel-by-voxel connectivity matrices. Here we present a series of analyses aimed at comparing the connectivity patterns derived using HTFA with those derived using other more commonly used approaches for studying connectivity in fMRI data (such as voxel-by-voxel functional connectivity analyses). We also describe steps that we have taken to maximize the computational efficiency of the hub-fitting algorithm.

Abstract: We present a novel feature extraction technique, Relative Brain Signature (RBS) that enables signifying differences between a finite numbers of populations. To evaluate our technique, we have used a set the EEG dataset of the “UCI Machine Learning Repository” of 77 alcoholics and 43 control subjects. For every subject, one RBS vector with respect to alcoholic and control populations were computed. An RBS vector denotes the relationship of a subject to one of the control or alcoholic populations. We employ the extracted RBS vectors to identify functional biomarkers over the cortical area of the alcoholics that had manifested distinct functional behavior in comparison to the control subjects. Moreover, we have evaluated the efficacy of the RBS vectors in correctly categorizing the subjects with respect to their original populations. To achieve this goal, a machine-learning algorithm to classify the subjects was employed. Subjects were correctly classified into alcoholic and nonalcoholic populations with accuracy up to 85%.

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Poster

542. Data Analysis

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MRC programme grant MR/J004146/1

Title: ParceNIP: parcellating neural images using PICo, a graded approach

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Abstract: In recent years, diffusion tractography has presented itself as a unique neuroimaging approach that allows large-scale parcellations of the cortex based on patterns of white
matter connectivity. While many approaches have been used to delineate distinct cortical regions, to date all parcellation schemes have focused on identifying discrete areas within the cortex that can be differentiated into hard clusters with clearly defined boundaries. However, there is evidence that this may not reflect the true underlying nature of the cortex, which instead may be better represented by a more graded approach with zones of transition instead of solid boundaries. We present a tractographic parcellation approach which allows for both graded and hard parcellations of the cortex. As an example of the approach we present a graded parcellation of the temporal lobe. A dataset containing diffusion weighted MR images from 24 healthy participants was used. Probabilistic tractography was performed from every voxel within the temporal lobe along the interface between the grey matter and white matter. From the output, a connectivity-based similarity matrix was obtained using the cosine similarity metric. A spectral reordering algorithm was then run on the laplacian of the similarity matrix, which reordered the voxels such that voxels with strong similarity were positioned close together within the matrix. Points that lie close together in the matrix were given similar intensities and back projected into brain space to generate a cortical similarity profile that automatically identifies the major axis of connective similarity across the cortex. The temporal parcellations produced were highly consistent across participants. They revealed a dorso-ventral and medio-lateral gradient consistent with current theories regarding the functional architecture of the temporal lobe. The dorso-ventral split potentially underlies the phonological and semantic divide in the temporal language network while the ventro-medial axis may underpin the memory and language divide in the temporal lobe.


Poster

542. Data Analysis

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 542.18/CC77
**Title:** Investigating default-mode subnetworks in autism with innovation-driven coactivation patterns


**Abstract:** Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by social deficits, communication impairments and restrictive, repetitive behaviors. Converging lines of evidence support the view that ASD is not a focal but a distributed disorder, in which abnormalities in the coordination of functional activity across large-scale networks give rise to core features and associated cognitive differences. Default-mode network (DMN) is the set of brain regions that are active during wakeful rest and are de-activated during attention demanding tasks, and is implicated in self-referential processing. It has been suggested that abnormalities in the DMN play a critical role in ASD. Whereas some studies that investigate the perturbations of brain activity during rest show increased DMN connectivity in ASD, others report a decrease. These studies have been hampered by several limitations such as failure to account for motion and small sample size as well as methodological issues; e.g. independent component analysis provides spatially independent networks and fails to fully characterize the complex spatiotemporal structure of brain function. The goal of the present work is to investigate the spatial and temporal alterations of the DMN in ASD. For that purpose, we utilized the innovation-driven coactivation patterns (iCAPs) method that can capture spatially and temporally overlapping networks. Resting state functional MRI was acquired from 51 ASD (ages 8-21 yrs) and 36 typically-developing (TD) participants (ages 8-25 yrs). In order to ensure data quality, the samples were matched for age, sex and motion; i.e., participants with head motion >1.5 SD above the sample mean were excluded (18 ASD, 3 TD). We identified the group specific iCAPs, and compared spatial alterations. The results were corrected for multiple comparisons using
Monte-Carlo simulations (p<.05). In the combined groups, we found three variations of DMN based on the involvement of the posterior cingulate, precuneus, and angular gyrus but with spatial variations in frontotemporal regions. The ASD group had greater activity in dorsal anterior cingulate and ventromedial prefrontal cortex and decreased activity in the right middle frontal gyrus relative to the TD group. These results provide evidence of altered coordination of activation in DMN in ASD using a well-matched sample. Future plans include investigating whether these differences are clinically relevant. Specifically, we will explore how the spatial and temporal variations of DMN activation relate to the severity of core features and cognitive dysfunction in ASD using neuropsychological measures and clinical ratings.


Poster
542. Data Analysis
Location: Hall A
Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM
Program#/Poster#: 542.19/CC78
Topic: G.07. Data Analysis and Statistics
Support: KAKENHI Grant No. 26350993
Title: Detecting activation patterns from functional MRI datasets with undetermined event onsets using support vector machines
Authors: *E. BAGARINAO, S. MAESAWA, H. WATANABE, H. ISODA; Brain and Mind Res. Center, Nagoya Univ., Nagoya City, Aichi, Japan
Abstract: Task-based functional MRI (fMRI) studies often rely on a priori knowledge of event onsets to identify activation patterns associated with the tasks. Detecting activation patterns in imaging studies where event onsets are unknown is very challenging. In this work, we proposed a method to detect activation patterns from fMRI datasets, even if event onsets are unknown, using support vector machines (SVMs). Neuroimaging datasets from 8 healthy volunteers (6 males/2 females, mean age = 21.62 years old) participating in a real-time fMRI study were used in the simulations. The data include a functional localizer scan, three feedback scans, and a test scan. All scans were designed in a block manner with alternating rest (30s) and task (30s) blocks. The tasks consisted of imagined finger tapping, word generation, and serial subtraction tasks. For this study, we used the datasets from the localizer scans to train SVMs to identify images belonging to each of the three tasks. The trained SVMs were then used to classify images from
the feedback and test scans. For each scan, a prediction time series for each task was obtained by assigning a value of 1 if the SVM predicted the task and 0 otherwise. Activation patterns associated with the tasks were generated by convolving the prediction series with the hemodynamic response function and using the convolved series as regressors in a general linear model analysis. The resulting activation maps were then compared to the one obtained using the actual onsets of the task blocks. We computed the spatial correlation between the two maps to quantify their similarity. Classification accuracies of the trained SVMs for the three tasks were about 91% on average. Correlation values of the generated maps were as high as 0.94, although cases with low correlation were also observed. On average, we got correlation values equal to 0.658 for imagined finger tapping task, 0.661 for word generation task, and 0.807 for serial subtraction task. These high correlation values implied that the obtained activation maps using SVM-derived prediction series closely resembled that obtained using actual onset timings. The primary advantage of the former is that it does not require knowing a priori the timing of the task onsets. Instead, it relied on the trained SVM to generate the needed regressors in constructing the corresponding activation maps. This is important as it would enable experiments where participants can freely switch from one task to the other and still be able to obtain the relevant activation maps. In particular, real-time fMRI studies in which the participant's real-time performance dictates the task to perform could benefit from this added flexibility.


Poster

542. Data Analysis

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 542.20/DD1

Topic: G.07. Data Analysis and Statistics

Support: Innoviris Brains Back to Brussels

Title: Time series analysis of pupillometric data

Authors: *A. ZENON; Inst. of Neurosci., Brussels, Belgium

Abstract: In addition to its response to light variation, the pupil diameter is also influenced by cognitive factors, such as cognitive workload, surprise or the exploration/exploitation trade-off and it is viewed as a proxy for noradrenaline release from the Locus Coeruleus. From a methodological point of view, a common issue with the analysis of the pupil size data is the
slowness of the pupil diameter variation, which prevents analyzing the pupil response related to fast-paced events. In a previous work addressing these issues, Hoeks and Levelt (1993) have proposed the use of a deconvolution technique to analyze the pupil size data. However, this method does not take into account the strong low-frequency spontaneous changes in pupil size or the high between-subject variability in the shape of the impulse response and in the delay between the event onset and the beginning of the response. Here we propose to use a system identification framework to optimize the analysis of pupillometric data. We first modeled the spontaneous changes in pupil diameter with an autoregressive moving average model (ARMA) and showed that using the residual from this model, or innovation error, uncovered subtle information from the data which was not accessible with standard techniques. We also modeled the responses to visual and auditory stimuli by means of exogenous inputs (ARMAX), allowing us to make inferences directly from the estimated parameters of the model. This approach was tested on a series of datasets involving different tasks. In conclusion, this new framework for the analysis of pupil size data improves significantly the sensitivity of the measure for subtle and/or fast-paced events.

Disclosures: A. Zenon: None.

Poster

542. Data Analysis

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 542.21/DD2

Topic: G.07. Data Analysis and Statistics

Support: The Sasakawa Scientific Research Grant

A Grant-in-Aid for Young Scientists (B)

Title: Cognitive function-based whole-brain parcellation using functional connectivity from voxels to regions labeled with cognitive terminology

Authors: *H. KURASHIGE*1,2, Y. YAMASHITA3, R. OSU4, Y. OTAKA2,5, T. HANAKAWA5, M. HONDA5, T. HISATSUNE5, H. KAWABATA5;

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Abstract: The brain is a complex system consisting of components with functional diversity. Our highly developed adaptability and creativity emerge from the combinations of such diverse functions. To understand the whole-brain information processing, therefore, we must resolve the brain into functional parts, know the computational characters of them, integrate the knowledge and construct the views for the whole brain as an integrated system. Whole-brain parcellation where the brain is decomposed into the interpretable components is important first step. While the parcellations based on structural and cytoarchitectural properties roughly correspond to functional segmentation of the brain, close investigation of brain functions has revealed the insufficiency and incorrectness of such anatomical parcellations. Recently, several researchers propose the ways to decompose the brain on the basis of the activity and connectivity. But the resultant parcels are often difficult to interpret. Here, we propose the novel way of whole-brain parcellation whose parcels are intrinsically labeled by the functionalities. First, using neuroimaging database Neurosynth, we assessed pseudo-activation maps for 109 cognitive functions (e.g., working memory and object recognition). From each map, mask for region of interest (ROI) corresponding to each cognitive function was generated. Then, resting-state functional connectivity (RSFC) from each voxel in whole brain to ROI masks were calculated. Thus we represent each voxel as 109 dimensional vector whose components are intensities of RSFCs. Applying clustering to the voxels, we obtained the whole-brain parcellation. It is noteworthy that all obtained parcels are intrinsically labeled by RSFCs to the ROIs corresponding to cognitive functions and are interpretable in a straightforward manner. Additionally, we analyzed the network whose nodes are obtained parcels and whose edges are RSFCs among parcels. Degree analysis revealed that the parcels strongly associated to the central executive (e.g. cognitive control and reasoning) tend to be at the influential positions in the whole-brain network. Centrality analysis revealed that parcels strongly associated to “search” and “mental imagery” are located at the highly separated position in the network. Moreover, we found that centrality is negatively correlated to the intensities of intra-ROI connectivity. In this study, we presented the highly interpretable whole-brain functional parcellation and showed the usefulness of it in the understanding of brain as an integrated system. More detailed systematic analysis based on the presented parcellation is a future direction.


Poster
542. Data Analysis

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 542.22/DD3
Abstract: Thalamocortical (TC) pathways were identified using high-angular resolution diffusion MRI (HARDI) tractography in postmortem fetal brains with no neurological histories ranging from 17 gestational weeks (GW) to 40 GW, as well as in vivo newborns to 28 years. Anterior, middle, and posterior TC pathways were segmented. No hemispheric asymmetry of the TC pathways was observed quantitatively during development. In fetal brains, pathways likely linked with neuronal migration to the immature thalamus structure were successfully imaged with regional differences in numbers and volumes. Evidence for protracted development in the anterior TC was observed both in fetal and postnatal data. We believe that the current study will be a useful reference for the normal TC development in future clinical imaging studies.
Authors: *Q. MENG*\(^1\), E. HONG\(^2\), F.-S. CHOA\(^1\);
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**Abstract:** Bistable illusion reflects two different kinds of interpretations for a single image, which is currently known as a competition between two groups of antagonism of neurons. Recent research indicates that these two groups of antagonism of neurons express different comprehension, while one group is emitting a pulse, the other group will be restrained. On the other hand, when this inhibition mechanism becomes weaker, the other antagonism neurons group will take over the interpretation. Since attention plays key roles controlling cognition, is highly interesting to find the location and frequency band used by brain (with either top-down or bottom-up control) to reach deterministic visual perceptions. In our study, we used a 16-channel EEG system to record brain signals from subjects while conducting bistable illusion testing. An extra channel of the EEG system was used for temporal marking. The moment when subjects reach a perception switch, they click the channel and mark the time. The recorded data were presented in form of brain electrical activity map (BEAM) with different frequency bands for analysis. It was found that the visual cortex in the on the right side between parietal and occipital areas was controlling the switching of perception. In the periods with stable perception, we can constantly observe all the delta, theta, alpha and beta waves. While the period perception is switching, almost all theta, alpha, and beta waves were suppressed by delta waves. This result suggests that delta wave may control the processing of perception switching.

**Disclosures:** Q. Meng: None. E. Hong: None. F. Choa: None.
Title: Thetaburst TMS to the right posterior superior temporal sulcus disrupts resting state connectivity across the face-processing network as measured with multi-echo fMRI

Authors: *D. A. HANDWERKER¹, G. IANNI², B. GUTIERREZ³, V. ROOPCHANSINGH², J. GONZALEZ-CASTILLO⁴, L. G. UNGERLEIDER⁵, P. A. BANDETTINI²,⁴, D. PITCHER³; ¹SFIM/LBC, ²FMRIF, NIMH, NIH, Bethesda, MD; ³SN/LBC, ⁴SFim/lbc, NIMH, Bethesda, MD

Abstract: Thetaburst transcranial magnetic stimulation (TBS) to the right posterior superior temporal sulcus (rpSTS) or the right occipital face area selectively disrupts BOLD responses within face-selective regions, both local and remote to the initial stimulation site (Pitcher et al., 2014). Given this modulation of task-evoked responses, we hypothesized that TBS delivered over the right posterior superior temporal sulcus would selectively disrupt functional connectivity within face-selective regions, as measured by resting state fMRI. Subjects participated in three separate fMRI sessions across different days. In the first, a high-resolution T1 weighted anatomical scan, as well as functional localization data were collected to identify individualized target sites for stimulation. The voxel within the rpSTS exhibiting peak activation using a contrast of faces greater than objects was identified for stimulation, as well as the anatomically defined hand-knob region (rHk) within the right primary motor cortex. In the remaining two sessions, multi-echo fMRI data were collected on a 3T GE MRI (TR=2s, TE=14.8, 27.1, & 39.5ms, 3mm³ voxels). Subjects completed two 10-min resting fMRI runs before and two runs after receiving TBS to the rHk or rpSTS. The order of site stimulation was counterbalanced across subjects. Both target sites were located using the BrainSight TMS-MRI coregistration system. A MagStim Super Rapid stimulator delivered TBS via a figure-eight coil with a wing diameter of 70 mm. The TMS coil handle pointed upwards and parallel to the midline. TBS was delivered at an intensity of 30% machine output over each subject’s functionally localized rpSTS or anatomically defined rHk. The stimulation paradigm consisted of 3 pulses at 50 Hz repeated at 200 ms intervals for 60 seconds. fMRI signal fluctuations that were unlikely to be due to blood oxygenation changes were removed using the multi-echo independent component analysis (ME-ICA) method (Kundu et al 2011). A mean time series was extracted from a sphere centered on the rpSTS stimulation site for each subject and each run and correlated to the rest of the brain. Areas with group correlation strength differences between the first run...
before and the first run after TBS were identified. Consistent with our hypothesis, TBS delivered over the rpSTS decreased connectivity to face-selective regions in the fusiform, middle occipital, and middle temporal gyri. There was no decrease in connectivity to these regions when TBS was delivered over the rHk. These results show that TBS to the rpSTS selectively decreases functional connections to face-selective regions even without task-evoked responses.


Poster

542. Data Analysis

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 542.25/DD6

Topic: G.07. Data Analysis and Statistics

Support: NIMH Intramural Research Program

Title: Using multi-echo cardiac gated fMRI to better denoise brainstem data

Authors: *J. Gonzalez-Castillo*, L. C. Buchanan, D. A. Handwerker, V. Roopchansingh, J. A. Derbyshire, B. E. Gutierrez, P. A. Bandettini;

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Abstract: Brainstem activity is difficult to detect with fMRI due to pulsatile motion. While cardiac gating (CG) can correct this by imaging the brainstem at the same relative position, resulting irregular repetition times (TR) induce variable baseline magnetization and hinder detection. In the past, a modeling approach to correct for such baseline shifts in CG-fMRI was proposed (Guimaraes et al. 1998). Here we evaluate a fully data-driven alternative based on Multi-Echo ICA (ME-ICA; Kundu et al. 2012), which can automatically separate BOLD (e.g., activations) from non-BOLD (e.g., T1-related fluctuations, hardware instabilities) fluctuations based on differences in TE-dependence. In principle, ME-ICA should automatically detect and remove CG-fMRI baseline fluctuations given their non-BOLD nature. To evaluate this possibility, we acquired gated and non-gated multi-echo fMRI data with an auditory block paradigm and focused our analysis in regions of the ascending auditory pathway, and particularly in the inferior colliculus (IC) located in the brainstem. The goal is to evaluate if CG-fMRI combined with ME-ICA can be used to reliably detect activation in the IC at the single-
subject/single-run level. METHODS: Four functional runs (2 gated, 2 non-gated) were acquired in each of 5 subjects (3T, FA=60°, TE=13.9/31.7/49.5ms, #Slices=33, #Acq=136, Res=3x3x3mm3, nominal TR=2.5s). Three different analyses were conducted. First, we produced activation maps using only the middle echo to reproduce standard fMRI analyses (SE). Second, analyses were conducted using an optimal combination of the echoes (OC; Poser et al. 2006), and ME-ICA denoised time-series. Finally, analyses were also conducted using the Guimaraes et al. modeling approach for comparison purposes. RESULTS: In CG-fMRI, activation within the IC is not reliably detected in the SE and OC analyses, yet after the use of ME-ICA activation was found within this region in all runs and all subjects. In non-gated data, activity in IC can be detected in many instances, yet detectability improves substantially in the OC and MEICA analyses. Regarding MEICA, the auditory task component was always marked as good. In CG-fMRI runs, a component that resembles a T1 map and correlates with TR shifts was always marked as noise. In addition, several rejected components correlated significantly with physiological and motion traces, confirming the denoising power of ME-ICA. CONCLUSIONS: ME-ICA can reliably capture and eliminate baseline signal fluctuations due to variable TR in CG-fMRI. Moreover, combined use of CG-fMRI and ME-ICA permits reliable detection of activity in the IC at the single-subject/single-run level.

questions: A. How far from the critical state does the brain operate, and B. How is that state maintained? A. Quantifying the distance to criticality $d$ can be biased due to subsampling, i.e. only a small fraction of neurons can be recorded in parallel [2,4]. We mathematically derived a novel estimator for $d$, which is reliable even under strong subsampling. The estimator is based on multiple linear regressions. Applying the estimator to spike recordings in vivo from rat hippocampus, cat visual cortex, and monkey prefrontal cortex consistently showed that the brain operates in a slightly subcritical regime with $d \sim 0.02$. This corresponds to an effective reduction in the mean excitatory synaptic strength $w$ of $\sim 2\%$ compared to criticality. B. What mechanism promotes self-organization to subcriticality? A natural candidate mechanism is homeostatic plasticity [5]. We studied the effects of spike rate homeostasis ($H$) on the distance to criticality in two types of networks (integrate and fire or stochastic point neurons). $H$ was implemented globally: The excitatory synaptic strength was initially set to a mean value $w$, and then updated proportionally to the difference between past spike rate $R$, and target spike rate $R_0 = 1$ Hz, using a slow time constant of $T = 1000$ s, as follows: $\frac{dw}{dt} = \frac{(R_0 - R)}{T}$. Indeed, $H$ assured that the networks assumed a slightly subcritical state, independent of the initial synaptic strengths. Notably, an increase in the external input (stimulus strength) altered the set-point of the network to a more subcritical state. Overall, the analysis of in vivo spiking activity across species, and the theoretical study of homeostasis suggest that the brain organizes itself to a subcritical state, not to criticality proper. We suggest that spike rate homeostasis is a key mechanism to maintain subcriticality. Compared to criticality, subcriticality implies a reduction in processing capability, but avoids instability. References 1. Beggs & Plenz (2003) 2. Priesemann et al., (2013) 3. Boedecker et al., (2012) 4. Priesemann et al., (2014) 5. Turrigiano (2012)

Disclosures: V. Priesemann: None. J. Wilting: None.

Poster

543. Data Analysis: Neuronal Networks

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 543.02/DD8

Topic: G.07. Data Analysis and Statistics

Title: Chronic administration of THC and SR141716-precipitated cannabinoid withdrawal in the rat brain: a complex network analysis

Authors: *G. SENTHINATHAN, G. WILLEMS, S. SKRZYP CZAK, C. LECKIE, P. E. MALLLET, B. E. MCKAY; Wilfrid Laurier Univ., Waterloo, ON, Canada
Abstract: The pattern of behavioral and neural changes associated with the chronic administration of Δ9-tetrahydrocannabinol (THC) suggests that tolerance develops quickly. Previously, we investigated neural activation among anatomically discrete reward-related brain areas in adolescent and adult rats by quantifying c-Fos immunoreactivity (Fos-IR) in response to: 1) acute SR141716 (a selective cannabinoid receptor antagonist), 2) chronic THC (10 mg/kg, IP, twice daily for 6 days), and 3) SR141716-precipitated cannabinoid withdrawal following chronic THC exposure (Senthinathan et al., 2014). To further analyze the Fos-IR data set, we have applied complex network analyses to assess the functional connectivity among these reward-related brain structures. For each group, Spearman’s rho for the Fos-IR data was calculated for all possible pair-wise combinations of the ~40 brain areas. The weight of the correlation coefficient indicates the strength of the functional relationship between a pair of structures. We found that chronic exposure to THC was associated with an increased number of functional connections in brain reward systems. Additionally, following chronic THC exposure, this increased number of functional connections seemed to be blunted by the administration of SR141716. An age-related difference was revealed by the analysis of the cluster coefficient (the main measure of the local structure of a network, defined as the ratio of the actual number of functional connections and the maximum possible number of connections). Among adult rats, chronic THC increased clustering. Chronic THC treatment did not seem to impact clustering among adolescent rats. Further, only among the adolescent rats, acute administration of SR141716 was associated with increased clustering. A rigorous set of complex network analyses will be applied to further investigate these functional neural networks. Applying the complex network analysis technique to c-Fos IR data sets brings forth new methods for visualizing and conceptualizing neural activation.


Poster

543. Data Analysis: Neuronal Networks

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 543.03/DD9

Topic: G.07. Data Analysis and Statistics

Title: The default mode network is spatially but not temporarily consistent

Authors: *E. SHOKRI-KOJORI1, D. TOMASI¹, N. D. VOLKOW¹²; ¹Natl. Inst. on Alcohol Abuse and Alcoholism, ²Natl. Inst. on Drug Abuse, NIH, Bethesda, MD
Abstract: Much attention in fMRI research has been paid to the default mode network (DMN), a set of parietal, temporal, and medial frontal regions that appear to fluctuate in synchrony at a slow rate (< 0.1 Hz) during rest and, occasionally, task states. It has been suggested that these fluctuations reflect spontaneous thoughts and mind wandering. While DMN has been reliably detected in many studies, the nature of the slow rate synchrony within DMN regions remains elusive. To shed further light on this phenomenon, we assessed spatiotemporal consistency of DMN within subjects. For spatial consistency, the reproducibility of voxels showing DMN-related activity was measured. For temporal consistency, the reproducibility of the frequency contents of the DMN time course was measured and its association with respiration time course was evaluated. Data from four high resolution (2-mm isotropic, TR = 0.72 sec) resting-state sessions, each 14.4 minutes long, on 260 subjects were included in this study. Spatial map and time course of DMN were extracted per session per subject using an automated independent component selection approach. Average-score intraclass-correlation (ICC) analysis was used to measure within-subject reproducibility of DMN spatiotemporal characteristics. We found that the location of voxels within DMN is highly reproducible within subjects (mean ICC = 0.79; Bonferroni, p < 0.05). For temporal characteristics, we found the 0.1-0.55 Hz frequency range was the only reproducible part of the total spectrum (0-0.69 Hz) within subjects (mean ICC = 0.40; Bonferroni, p < 0.05). But the 0-0.1 Hz frequency range, accounting for about 90% of the power of DMN time course, did not show consistent rates of fluctuation within subjects (mean ICC = 0.10). We further hypothesized that the reproducible part of DMN spectrum corresponds to respiration rate. Additional ICC analysis on coherence between respiration and DMN time courses supported this hypothesis and showed significant reproducibility in the association between the two spectrums in 0.15-0.5 Hz frequency range (mean ICC = 0.46; Bonferroni, p < 0.05). In summary, we report that brain regions related to DMN are spatially consistent within subjects. Yet, the main rates at which these regions fluctuate together (within 0-0.1 Hz) is not reproducible within subjects across sessions. We conclude that the high-power low-frequency content of DMN time course may not be an index of systematic slow rate fluctuations in neuronal populations and may be confounded by a complex of physiological factors such as respiratory rhythms and characteristics of the vascular system, as well as fMRI aliasing artifacts.


Poster

543. Data Analysis: Neuronal Networks

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 543.04/DD10
Title: Quantifying small-worldness in weighted brain networks: small-world propensity

Authors: S. E. F. MULDOON¹, E. W. BRIDGEFORD¹, *D. S. BASSETT²;
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Abstract: Brain networks have repeatedly been shown to have small-world properties, with high local clustering yet short average path length between any two nodes. Recently, the small-worldness of the brain has been challenged by the observation that new data from structural brain networks indicates a high level of connectivity between brain regions, making the network too dense to have small-world properties. However, this claim is based on traditional measures for assessing small-worldness that do not take the weighted nature of connections between brain regions into account. By relying on analysis of binary networks, the network density is artificially inflated, and the fact that strong and weak connections will differentially contribute to the overall network structure is ignored. Additionally, the fact that these measures are dependent on network density, makes comparison of brain structure between different groups (e.g. diseased versus healthy) or different task states difficult. We therefore present a novel diagnostic of small-world structure called the Small-World Propensity (SWP) that quantifies small-world structure in a density independent manner and can be applied to both weighted and binary networks. We apply this new measure to a variety of structural and functional brain networks and show its usefulness in quantifying small-worldness in order to gain insight into differences between network organization across systems. While all brain networks examined show small-world characteristics, surprisingly, the neuronal network of *C. elegans*, the original example of a biological small-world network, shows the least amount of small-world structure.


Poster

543. Data Analysis: Neuronal Networks

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 543.05/DD11

Topic: G.07. Data Analysis and Statistics
Title: Analysis of motifs' spontaneous and evoked dynamics in patterned cortical neuronal networks

Authors: *M. BISIO*¹, Y. PIASETZKY², M. OLIVEMBOIM², S. KANNER³, M. CHIAPPALONE¹, P. BONIFAZI²
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Abstract: Cultured neuronal networks cultivated on Micro Electrode Arrays (MEAs) are a widely used tool for the investigation of neuronal network mechanisms, providing structural framework for long term recordings of electrophysiological activity, as well as the response to electrical stimulations. Patterned networks are composed of distinct functional modules with highly connected neurons within the modules and sparse connectivity between them. The structure of each module can be treated as a uniform network, with all neurons in the modules firing in a quasi-synchronized way, while the whole network can perform more complex patterns of temporal activity that propagate from one module to another. The occurrence of Network Events (NEs), i.e. “network motifs”, composed by a specific temporal sequence of activation between the modules or electrodes has been investigated. The used experimental protocol to consists of the following steps: i) 1-hour recording of spontaneous activity; ii) a stimulation session, which consists of stimulating one electrode of each module using a train of 50 stimuli (0.2 Hz); iii) 1-hour recording of spontaneous activity. Overall, 18 patterned networks have been recorded. The analysis can be carried out both at single electrode and at single module resolution, during both spontaneous and evoked activity. The single electrode resolution provides a higher spatial resolution including also the temporal activation within each module. The single module resolution aims at highlighting the connections between the modular structures and the inter-module activity propagation. We tested the hypothesis that consecutive NEs display higher similarity than randomly played NEs, namely, patterned networks have a "dynamical working memory" state which persists on specific motifs which share high similarity. So, the average similarity between pairs of NEs has been reported as a function of the inter-NE intervals. In particular, it has been found that, in spontaneous conditions, motifs have an inter-NE distances distribution which is significantly different from a Poissonian distribution, i.e. obtained when motifs are randomly played. Then, it was also observed that at the evoked activity level the average similarity’s profile computed between the different stimulation trials is not significantly different from a Poisson distribution. This allows to conclude that stimulation induces a deterministic response, i.e. similar motifs are observed after each stimulation trial. These results constitute an important step in investigating possible ‘memory’ processes arising in cortical networks in vitro.

**Title:** On the use of electrophysiological signatures in translational research

**Authors:** *R. G. PORT*¹, S. J. SIEGEL², G. C. CARLSON³, T. P. L. ROBERTS³;  
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**Abstract:** While there has been great progress researching neurodevelopmental disorders such as Autism Spectrum Disorders (ASD); findings (ranging from protein structure to whole brain connectivity) are often difficult to integrate into a compressive theory about the biological basis underlying such disorders. Taking ASD as an exemplar, the use of electrophysiological signatures as ‘biomarkers’ (biologically based markers) exhibits great promise, with signatures (M100 delay and Gamma-band (30-100 Hz) dysfunction) being observed repeatedly across both clinical populations and pre-clinical models. In addition, these biomarkers provide a convergence point for the different perturbations seen across multiple levels of study for ASD. Here we highlight the promise of these biomarkers in unifying electrophysiological findings across multiple modalities. Using the Matlab toolbox Fieldtrip, data spanning multiple levels of study (clinical MEG, pre-clinical EEG and in-vitro electrophysiology (lfPs and VSDi)) were analyzed utilizing near identical analysis routines.

**Disclosures:** R.G. Port: None. S.J. Siegel: None. G.C. Carlson: None. T.P.L. Roberts: None.
Abstract: The brain is a highly non-uniform organ that consists of a complex neural network, where neurons communicate with each other through within- and between-area axonal projections. In the past decades, neurophysiological studies using single unit recording (SUR) in primates have revealed relationships among neuronal activities, cross-area anatomical connectivity, and cognitive behaviors. Unfortunately, however, the number of simultaneously observable brain regions with SUR is usually limited to less than ten. Although many innovative technologies, including multi-electrode array recording, are expanding the recordable spatial scale, it is currently difficult to record the brain-wide activity at single-cell resolution in primate. On the other hand, recent wide-field electrocorticogram (ECoG) complements the limitation of SUR, by simultaneously observing the electrophysiological signals at the scale of entire cortex. However, since the ECoG signal reflects activity of neural population rather than single cells, its relevance to the previous SUR studies needs to be clarified. To fill this gap, we compared the cross-area signal propagation in ECoG data from the whole cortical surface in macaque monkey (Nagasaka et al., 2011) with spike delays reported in a number of previous SUR studies, as well as with the large-scale anatomical network (Lewis & Van Essen, 2000). Our preliminary results suggest that (1) the time lag of cross-correlation peaks in ECoG signal predicts spike delay in cross-area information propagation, (2) the both time delays can be accurately predicted by path length in anatomical connectivity network rather than physical distances among areas, and (3) the correlation between anatomy and ECoG signal propagation delay are modulated by the arousal levels of monkey. These results demonstrate that ECoG signal preserves the temporal information of single-unit spike propagation across areas, as well as that effective cross-area network switches depending on the brain state.
Abstract: Administration of delta-9-tetrahydrocannabinol (THC) results in a wide array of behavioural effects via its action on the brain’s endocannabinoid (eCB) system. The current study examined the effects of THC on neural activation throughout the brain using complex network analysis, which is a relatively novel method of analysis that permits the quantification of functional connectivity in large neural networks, clusters of activity, and individual regions. Rats were injected with 5 mg/kg THC, or its vehicle, and then transcardially perfused two hours later. Brains were sectioned and immunohistochemistry was used to label Fos—the protein product of the immediate-early gene c-fos. Neural activity was quantified by counting immunoreactive nuclei in a total of 81 brain regions. Functional connectivity matrices were generated for each group and
significant connections were analyzed. THC administration resulted in increased number, strength, and clustering of functional connections across the network. Further analyses will investigate the properties of network connections and clusters of activity. These results provide novel information on the neural effects of THC administration, as well as on the use of complex network analysis in behavioural neuroscience. Support for this research was provided by NSERC and Wilfrid Laurier University.


Poster

543. Data Analysis: Neuronal Networks

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 543.09/DD15

Topic: G.07. Data Analysis and Statistics

Support: Tinnitus Research Consortium

Title: Unsupervised hierarchical clustering of resting state functional connectivity data to identify patients with mild tinnitus

Authors: S. A. SCHMIDT1, M. SCHUBEL1, A. N. HIRANI1, Y. BARYSHNIKOV1, *F. T. HUSAIN2;
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Abstract: In this study, unsupervised hierarchical clustering of resting state functional connectivity data (from Schmidt et al, PloS One, 2013) was performed to separate tinnitus patients from controls. Hierarchical clustering on tinnitus patients using neuroimaging has not previously been employed, but has the potential to identify subgroups within the heterogeneous tinnitus population. Such methods may also clarify the discrepant results seen in multiple studies of resting state functional connectivity in tinnitus patients. Clustering was performed on correlations between the average time courses between voxels. Different time windows (20, 40 and 60 second in addition to the full five minute data) were used when averaging the data to examine any short-term effects. Data were clustered in two forms: directly as preprocessed fMRI data, and also as weighted graph Laplacian eigenvectors. For the eigenvector data, three different distance cutoffs (5, 10 and 20 mm) were used to weight the edges of the constructed graph Laplacian. Finally, correlations were examined across the whole brain as well as in sets of regions of interest selected based on the results of our previous study (Schmidt et al, PloS One,
2013) and included regions of the default mode, dorsal attention, auditory networks and the
limbic system. The number of clusters was fixed at two, as we were interested in dividing
subjects into two groups: tinnitus and non-tinnitus. The non-tinnitus group consisted of either
normal hearing or hearing loss controls. Clustering success was determined via calculation of the
Rand index. In general, clustering was more successful when separating tinnitus patients from
normal hearing controls using regions of interest; this produced the best Rand index of 0.80
when Ward linkage hierarchical clustering was applied to correlations in regions of interest
located in auditory and limbic regions. Our results suggest that unsupervised hierarchical
clustering can successfully differentiate tinnitus patients from controls based on their
connectivity patterns. The technique used here will also be applied to different tinnitus
subgroups, in particular groups of differing tinnitus severity, to assess the sensitivity of the
technique in organizing heterogeneous patient groups.

Disclosures: S.A. Schmidt: None. M. Schubel: None. A.N. Hirani: None. Y. Baryshnikov:
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Poster

543. Data Analysis: Neuronal Networks

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Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 543.10/DD16

Topic: G.07. Data Analysis and Statistics

Title: Assembling the multimodal, multidimensional brain network

Authors: *M. Y. MAHAN*¹, A. P. GEORGOPOULOS²;
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Abstract: The majority of brain connectivity studies focus on either structure or function, while
a few studies aim to unite them. Unfortunately, these combination studies fall short of true
multimodal integration and instead provide comparisons of networks across modalities.
Although meaningful insights have been gathered from these studies, the development of a
comprehensive network that describes both brain structure and function simultaneously is still
missing. For that reason, a novel multimodal, multidimensional network was derived. In
addition, both spatial and temporal components are incorporated in order to capture the innately
spatiotemporal properties of the brain. Structural (sMRI and DTI) and functional (fMRI and
MEG) data were collected from 65 cognitively healthy women (32-74 years old). Graph
construction is based on a combination of previous work whereby multiple graph construction
methods were examined. First, the number nodes in the graph were generated randomly and
labeled with sMRI measures of gray matter volume, thickness, and surface area. Next, multiple edges were defined from the remaining data, namely, (1) DTI measures of white matter tractography, (2) fMRI measures of correlations, and (3) MEG measures of correlations. Taken together, the measures are incorporated into one multidimensional graph characterizing structural and functional connectivity. For the multimodal, multidimensional graph, characteristic network measures were calculated and compared across the sub-networks. In addition, graph theory algorithms were adapted and used to calculate network measures from each area, lobe, hemisphere, and whole brain, resulting in a comprehensive network assessment for each subject. Using these results, the effect of age was calculated for each network metric. Furthermore, spatiotemporal data mining approaches, informed by previous results, were used to capture spatiotemporal processes of brain connectivity patterns. Taken together, the results are used to construct a model of how brain communication patterns change with age, in such a way that brain function remains healthy.

Disclosures: M.Y. Mahan: None. A.P. Georgopoulos: None.

Poster

543. Data Analysis: Neuronal Networks

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 543.11/DD17

Topic: G.07. Data Analysis and Statistics

Support: NIH Grant DA038009

Title: Comparing MDPV’s and cocaine’s induced modulation of resting state networks

Authors: *L. M. COLON-PEREZ¹, M. FEBO²;
¹Psychiatry, ¹Univ. of Florida, Gainesville, FL

Abstract: Drug abuse has detrimental effects on the brain that lead to drug use disorders. In vivo non-invasive biomarkers are needed to determine the neurobiological outcomes of addictive drugs on the brain. Analysis of functional brain network organization using graph theory measures offers a robust and objective analytical approach to address this need. It is anticipated that this could provide a useful biomarker of drug action in the brain of animals and humans for neuroimaging studies. In the present study we determined the effects of two potent and addictive psychostimulant drugs, 3,4-methylenedioxypyrovalerone (MDPV) and cocaine, on various graph measures of network connectivity. MDPV is a designer cathinone drug that is present in formulation of street drugs known as ‘bath salts’. It shares a similar mechanism with cocaine,
inhibiting the reuptake of dopamine (DA), but with a greater affinity for the DA transporter.
Resting state functional magnetic resonance imaging (fMRI) datasets were collected 1 h after i.p. administration of 1.0 mg kg⁻¹ of MDPV, cocaine of 15 mg kg⁻¹, and a control group administered with saline. To determine the differences of MDPV and cocaine induced alterations in connectivity we compared network relevant measures (i.e. degree, path length, and clustering coefficient) to controls. Images were processed for seed-based functional connectivity analysis using a segmented atlas of the rat brain. By modifying the threshold that generate networks for each group (Cocaine = 0.1, MDPV = 0.1 and control = 0.4) graph densities were maintained the same (25% of all possible pairs) for all groups in order to avoid differences in connectivity due to graph density differences. We observed a reduction in degree, indicator of nodal connectivity with the entire network, among cortical amygdala and ventromedial striatum, while lateral amygdala and infralimbic cortex in response to both cocaine and MDPV. The global characteristic path length did not show any difference between control and drug treated groups. Overall the clustering coefficient, an indicator of network integration, was reduced for the accumbens core and shell and ventromedial striatum for both drug groups. These results suggest that the brain under the effects of drugs maintain efficient information transmission through short path lengths; however, their integration is affected by reducing the clustering of local communities. This suggests a mechanism of disruption of local information processing in key areas of the brain (e.g. accumbens). The long-term objective of the present work is to develop a useful approach for distinguishing the network-level effects of addictive drugs.

Disclosures: L.M. Colon-Perez: None. M. Febo: None.

Poster

543. Data Analysis: Neuronal Networks

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

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Topic: G.07. Data Analysis and Statistics

Support: ICT-FET FP7/2007-2013 #284772

Title: Interfacing in silico and in vitro neuronal assemblies: relevance of electrical stimulation temporal distribution on neural network responses

Authors: *M. CHIAPPALONE¹, V. PASQUALE², P. NOWAK³, P. MASSOBRIO³, A. BRUZZONE¹, F. SCARSI², J. TESSADORI²;
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Abstract: Electrical stimulation has been in use for decades as a tool to elicit activity in neuronal systems, both in vivo and in vitro: traditionally, the most common method to probe neural activity has been the delivery of regular stimulation, with fixed frequency and amplitude. While it is a well-known fact that input variability is fundamental in single neuron responses, the effects of variable input sequences at the network level is a seldom investigated subject. Within this work, we studied the capability of in vitro neural networks to follow input stimulations with an increasing degree of regularity. Furthermore, we interfaced an in silico neuronal network to an in vitro one, with the ultimate goal of performing a closed-loop bidirectional connection in future studies. As neural substrates, we used cortical cultures dissociated from embryonic rats (E18) and kept alive over Micro Electrode Arrays (MEAs) for 3-4 weeks. Our stimulation sequences consisted in identical stimuli with different inter-stimuli intervals (ISIs): the ISIs in our experiments followed a distribution, with regularity increasing with the value of $\beta$ (values tested were 0, 0.5, 1, 1.5, $\infty$). Comparisons occurred on the correlation between low-passed (rectangular window, 0.1s in length) stimulation and network-wide spike trains. In silico networks were interfaced to biological ones through their network bursts: stimulus delivery was triggered by the detection of a ‘network burst’ in the in silico network. We analyzed evoked network responses in order to evaluate whether biological activity correlates with stimulation. Our results show that cultures are largely unable to synchronize network-wide responses with regular stimulation at the considered stimulation rate (0.5Hz), while this occurs to a much higher degree for irregular stimulations.


Poster

543. Data Analysis: Neuronal Networks

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 543.13/DD19

Topic: G.07. Data Analysis and Statistics

Support: JSPS KAKENHI 25120010

JSPS KAKENHI 25730147

JSPS KAKENHI 25610102

Title: Extracting non-linear spatiotemporal dynamics in active dendrite: data-driven statistical approach
Recent findings suggest that active dendrite contributes more important roles in neural information processings such as directional selectivity [Sivyer and Williams, Nature Neurosci., 2013], integration of sensory and motor inputs [Xu et al., Nature, 2012], and so on. For example, recent experimental results showed that active dendritic computation plays a key role in directional selectivity for visual stimuli in retinal ganglion cells [Sivyer and Williams, Nature Neurosci., 2013]. However, the mechanism of active dendritic computation is still unclear, since the spatiotemporal dynamics of active dendrite remains unknown. Great advances in measurement technology enable us to deal with spatiotemporal data from neural systems including dendrites as imaging data. However, the observable information in the measurements are limited, compared with the complexity of the entire neural system. Some estimation techniques are proposed using the state space modeling approach to extract the spatiotemporal dynamics of the dendrites. In some of previous methods, only membrane potentials are estimated while assuming the parameters underlying spatiotemporal dynamics are known, and most of previous methods only consider the estimation of linear dynamics in multi-compartment models or nonlinear dynamics in single-compartment models [Paninski, 2010, Omori et al., 2013; Omori, 2014], although it is important to establish nonlinear dynamics for spatiotemporal membrane evolution in multi-compartment models to reveal the active dendritic computation. In this study, we propose a statistical method to estimate nonlinear spatiotemporal membrane dynamics of active dendrites in order to extract nonlinear dynamics of dendritic membrane. We employ a framework of probabilistic information processing to extract the nonlinear spatiotemporal dynamics from partially observable data. First, we formulate generalized state-space model of active dendrite, based on multi-compartment model. Next, sequential estimation algorithm is derived for the generalized state space model. By employing sequential Monte-Carlo method and other statistical methods, membrane dynamics and its underlying electrical properties are simultaneously estimated in the proposed method. Using the proposed method, we show that nonlinear spatiotemporal dynamics in active dendrites can be extracted from partially observable data.
Title: A comparison of single and multi-shell diffusion-weighted MRI imaging in the anesthetized macaque for thalamocortical tractography

Authors: *K. BROWN*, R. A. SHEWCRAFT, P. VELASCO, B. PESARA; 1Ctr. for Neural Sci., 2Ctr. for Brain Imaging, New York Univ., New York, NY

Abstract: White-matter tractography using diffusion-weighted MRI imaging (DWI) is widely used to study anatomical connections in the primate brain. Recent advances in multi-shell imaging sequences have promised greater accuracy in reconstructing anatomical pathways. However, a direct comparison of single and multi-shell methods for tractography across a single non-human primate in vivo has not been reported. Here, we compare the efficacy of single-shell and multi-shell acquisition schemes for in vivo macaque DWI tractography. To judge the effectiveness of each scheme, we reconstructed thalamocortical projections using a probabilistic tracking method designed to distinguish crossing fibers, segmented the thalamus according to the most likely cortical target, and compared this classification to known thalamic subdivisions. Each subject was anesthetized with a constant IV infusion of 0.5mg/hr/kg of atracurium and 4mg/hr/kg of sufentanil, or isoflurane alone and scanned in the sphinx position. Data were acquired with a 3T Siemens Allegra (Erlangen, Germany) using 3 elements of a 4-channel phased array (Nova Medical Inc., Wilmington, MA), in a 2D single-shot twice-refocused DW-SE-EPI sequence, with 64 gradient directions in 1.2 mm² in-plane resolution (TR = 7000ms; TE = 110ms; B-values: 0, 750, 1500, 2250 s/mm²; FOV: 80x72 pixels; slices: 45; slice thickness: 1.2 mm; DWI to b0 ratio 65:1). To correct geometric distortions from field inhomogeneities caused by the non-zero off-resonance fields, the runs for each b-value were collected twice, one with reversed phase-encode blips, forming pairs of images with distortions going in opposite directions. For each b-value, we collected an extra pair of images without diffusion gradients and with a TE of 74ms, but otherwise identical to the DWI. From these pairs the off-resonance field was estimated and used to combine the DWI pair into a single corrected one. A total of 21 pairs were recorded. Eddy currents were subsequently corrected for using FSL’s eddy tool. We modeled diffusion for each voxel as up to two crossing fibers using a resampling procedure and obtained path distributions using FSL’s bedpostX and probtrackx tools. Recent observations suggest that communication across cortex depends on intermediate thalamic relay sites. As a result, correct identification of anatomical pathways depends not only on resolving cortico-cortical tracts, but constituent thalamic relay tracts as well. Our data hold implications for in vivo approaches to identify macrocircuits for same-subject functional imaging and electrophysiological recording.

Title: Testing the statistical significance of dynamical structure in neural population responses

Authors: *G. F. ELSAYED¹, M. T. KAUFMAN³, S. I. RYU⁴, K. V. SHENOY⁴, M. M. CHURCHLAND¹, J. P. CUNNINGHAM²;
¹Neurosci., ²Statistics, Columbia Univ. In the City of New York, New York, NY; ³Cold Spring Harbor Lab., Cold Spring Harbor, NY; ⁴Stanford Univ., Stanford, CA

Abstract: Many hypotheses consider the role that population dynamics play in the computational structure of various neural systems. One common question is whether there exist consistent population dynamics across different experimental conditions. To illustrate this, consider the joint neural responses of $N$ neurons to each experimental condition, which can be viewed as a point moving over time forming a trajectory through $N$-dimensional neural space. In neural spaces, dynamics are modeled as a single flow field that governs the evolution of many neural trajectories. Here, we test for the existence of consistent dynamics, defined as a single fixed dynamical rule that governs the evolution of neural trajectories for all conditions. In particular, we are interested in the basic question: do neural data show any dynamical consistency beyond what would be expected by non-dynamical 'null' data? The main difficulty in addressing this question is that the notion of null data is ill-defined. For example, white noise is a null dataset that may make any collection of neural data look dynamically consistent in comparison, due to the surface features of real data such as temporal smoothness, across-
condition smoothness, and pairwise neural correlations. Thus, the central challenge is to create null data with similar surface features to the real data. Here, we demonstrate procedures to create surrogate (null) data that preserve the surface features of the real neural data. To generate these surrogate datasets, we use ideas from permutation tests, which assume exchangeability of conditions under null hypotheses, to disrupt any dynamical consistency across neurons in real data. More importantly, via permutation techniques and matrix optimization methods, we enforce exchangeability of the surface features. If a dataset truly follows dynamical systems rules, it should be distinguishable from these surrogate datasets even though both share the same surface features. These permutation tests provide a conservative statistical test for dynamical structures and give guidance as to the number of neurons, conditions and time points needed to provide statistical power to these tests. As a first application, we test for the dynamical consistency of data from the motor cortex. The results show significant dynamical structure in the motor cortical population responses ($R^2 = 0.6$, $p < 0.001$), providing statistical support to recent hypotheses [Churchland & Cunningham et al., 12].


**Poster**

*543. Data Analysis: Neuronal Networks*

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 543.16/DD22

**Topic:** G.07. Data Analysis and Statistics

**Support:** LOEWE Grant "Neuronale Koordination Forschungsschwerpunkt Frankfurt (NeFF)"

**Title:** One man's prediction is another man's error - quantifying predictive coding at the retinogeniculate synapse independent of the observer's assumptions

**Authors:** *M. WIBRAL*¹, D. RATHBUN², W. M. USREY³, A. BASTOS⁴, P. WOLLSTADT⁵; ¹MEG Unit, Brain Imaging Ctr. Frankfurt, Frankfurt a.M., Germany; ²Inst. for Ophthalmic Res., Eberhard Karls Univ., Tübingen, Germany; ³Dept. of Neurol., Univ. of California Davis, Davis, CA; ⁴Picower Inst. for Learning and Memory, MIT, Boston, MA; ⁵MEG Unit, Brain Imaging Ctr., Goethe Univ., Frankfurt a.M., Germany

**Abstract:** Predictive Coding Theory (PCT), the idea that brains exploit statistical regularities to facilitate perception based on noisy sensory evidence, has become a successful paradigm in organizing empirical findings in neuroscience. Exploiting regularities can happen either by
passing on sensory evidence matching internal predictions (reliability coding), or by passing on sensory evidence not matching predictions (error coding). If we knew which strategy the brain used, we could objectively decide from neural data whether sensory input results in an error or a confirmed prediction. Conversely, if we knew exactly when an error should arise, we could decide from neural data which strategy the brain used. Without this knowledge, the assignment of neural signals to errors or confirmed predictions depends on the observer's view on what the system predicted - a circular approach. This problem is amplified at the level of cells, as predictions there can only relate to statistics of incoming spike trains. Thus, a cell's predictions are remote from semantics imposed by the observer. Moreover, cells in a brain that globally supports reliability coding might still locally code errors. Therefore, we need a way to state the type of coding without semantics. This can be done using local information dynamics. In this framework, local active information storage (LAIS) measures the predictable information in a time series, while local transfer entropy (LTE) measures the information passed to the next cell. Evaluating the correlation between the two distinguishes reliability coding from error coding.

We applied this framework to spike train recordings from 17 pairs of retinal ganglion cells (RGC) and lateral geniculate nucleus (LGN) cells in the anesthetized cat. We computed LAIS and LTE using nearest neighbour estimators from TRENTOOL/JIDT on spike trains convolved with synthetic post-synaptic kernels. We found a significant correlation of LAIS and LTE in 14/17 pairs \( (p<0.05) \). Of these, 8 had a positive LAIS-LTE correlation, indicating reliability coding, while 6 had a negative correlation, indicating error coding. The LAIS-LTE correlation in turn was strongly positively correlated \( (r=0.79; p<0.0002) \) with the contribution of an RGC input cell to the LGN cell's output, suggesting that the main retinal inputs to LGN cells are passed on when reliable, whereas secondary ones are passed on when surprising. We believe that our framework will improve the understanding of PCT at the circuit level.

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**Poster**

**543. Data Analysis: Neuronal Networks**

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**Topic:** G.07. Data Analysis and Statistics

**Support:** UNAM-DGAPA-PAPIIT-IN204014

**Title:** Clustering Analysis of Crayfish Agonistic behavior
Abstract: Agonistic behavior results in the establishment of a hierarchical order of dominance-submission which determines access to resources and remains stable in time. Agonistic interactions can be divided into two types: positive contacts, all actions that a particular crayfish exerts on its conspecifics, and negative contacts, all actions that a particular animal receives from the other. Positive contacts are: threat, attack and fight; negative contacts are: retreat and avoidance. The first purpose of this work was to study agonistic interactions by clustering analysis, in an attempt to determine what are the contacts that have the greater impact on the establishment of the social hierarchy. We used male crayfish, Procambarus clarkii, organized in triads whose weight and size differ by less than ten percent. We analyzed agonistic interactions by clustering analysis, which consists in a mathematical algorithm used to classify data. It calculates similarities and dissimilarities and organizes data in groups according to these properties. In establishing the social hierarchy, positive contacts occur most frequently in the first few days, while the negative contacts are presented markedly in the last days. Results show that when forming groups with three agonistic contacts, if we choose a negative contact and two positive contacts, groups are more defined than if we choose two negative contacts and one positive contact; the contacts that allowed us to identify more clearly the groups were: threat, attack and retreat. The second purpose was to determine the effects of interference experiments (interaction with unfamiliar animals) using clustering analysis. Results showed a displacement of groups whose magnitude and direction depends on the original social status of a given crayfish.

Authors: *M. SHIMONO*1,2;
1Harvard / MGH, Masanori Shimono, Boston, MA; 2Indiana Univ., Bloomington, IN

Abstract: Our brain is an organization based on highly non-uniform networks. The connectivity pattern generates another “closeness” which is more than the simple idea of short anatomical distances, and it is spoken that the non-uniform pattern helps us to understand the high computational efficiency etc. of the brain [Bullmore, Sporns, 2012]. In graph theory, when characterizing the network organization, there are two clear distinct characteristics, “weighted” and “non-weighted” networks. In macroscopic brain networks, the weights indicate relative strengths of fiber bundles connecting brain regions, and the strengths are not considered in non-weighted networks. This study focuses on relationships between “weights (strengths)” of connections between pairs of brain regions and “connectivity patterns” surrounding the respective brain regions. This comparison is currently a critical topic to understand brains of higher primates such as monkeys because, currently, all neuroscientists in the world do not have complete connectivity data including “weights” of high primate brains. Therefore, at this moment, in order to reach a better understanding of brains of higher primates, we need to complement information from both of “non-weighted networks covering whole brain regions” and “weighted networks covering partial regions”, and use them synergistically (Fig. A). Better understandings of high primates help us to understand detailed brain processes, this is what non-invasive technologies cannot capture. This knowledge could eventually help us to determine “What is uniquely human” in evolitional streams. From these backgrounds, this study compared weighted networks provided by Drs. Markov and Kennedy et al. [Markov et al., 2011] and non-weighted networks [Kötter, 2004; Bakker et al., 2012] as used in our previous report [Shimono, 2013]. This comparison declared several general principles between “weights” of connections between brain regions and “connectivity patterns” surrounding the brain regions (Fig. B), which should be representing information processes in the brain.

Disclosures: M. Shimono: None.
**Title:** Definition of sensory-evoked functional area in optical intrinsic signal imaging using a segmentation method

**Authors:** *C. YEON, D. KIM, H. CHOI, K. KIM, E. CHUNG; DMSE, GIST, Gwangju, Korea, Republic of*

**Abstract:** Optical intrinsic signal imaging (OISI) is a wide-field label-free functional brain optical imaging method that has been used to define functional brain area as it provides high spatiotemporal resolution. Single-color OISI has been used for simple functional area definition combined with other functional brain imaging modalities, meanwhile, multi-color OISI for tracing hemoglobin concentration changing and flow. Especially, single-color OISI takes quite simple experimental procedures thus, making it advantageous for stable baseline conditioning. However, signal processing methods to define sensory-evoked response area is not well established. In this work, we demonstrated single-color OISI system and segmentation method for quantification and simple definition of functional mouse brain area in the sensory area. We used Olympus BX51WI microscope body combining with Andor NEO sCMOS camera and 625 nm LED. Cranial window models (C57BL/6 male mice, n = 5) were used for brain optical imaging. We set pre-stimulation state as a baseline period and bipolar current stimulation state as a stimulation period. We calculated a ratio of two representative images, baseline and stimulation periods. We converted a single ratio feature of each pixel to CIELAB coordination to expand three-dimensional features. Then we calculated similarity and dissimilarity to find segmented regions based on the quantitative differences through features of pixels with a simple linear iterative clustering (SLIC) algorithm. Finally, we obtain the distinguishable area under the stimulation. We defined hind paw sensory areas from 5 cranial window mouse models. The hind paw area was distinguished into core and periphery area by OIS intensity. We applied a novel and simple segmentation method for our purpose of sensory brain area definition by using single-color optical intrinsic signal (OIS) that is reproducible and applicable to individual mouse and effectively distinguish the sensory-evoked functional area of hind paw. Fig. 1. (Left) White light image of the left hemisphere. (Right) segmented brain area.
Disclosures:  
C. Yeon: 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.; Institute of Medical System Engineering (iMSE) grant in GIST, The GIST-Galtech Research Collaboration Project through a grant provided by GIST in 2015. D. Kim: None. H. Choi: None. K. Kim: None. E. Chung: None.

Poster

543. Data Analysis: Neuronal Networks

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Title: Weighted wavelet z-transform on the uneven event timing of neuronal spikes

Authors: *W.-Y. WU¹, J.-J. HUANG², Y.-T. LIN³, P.-C. SHAO⁴, C.-T. YEN¹, M.-L. TSAI³, H.-W. TSAO³, R.-S. CHEN⁴, C.-C. YEN⁷;¹Dept. of Life Sci., Natl. Taiwan Univ., Taipei, Taiwan; ²Grad. Inst. of Electronics Engin., Taipei, Taiwan; ³Grad. Inst. of Communication Engin. Col. of Electrical Engin. and Computer Sci., Taipei, Taiwan; ⁴Dept. of Mathematics, Natl. Central Univ., Jhongli, Taiwan; ⁵Dept. of Biomechatronic Engineering, National. Ilan Univ., Ilan, Taiwan; ⁶Dept. of Life Science, Tunghai Univ., Taipei, Taiwan; ⁷Dept. of Mathematics, Fu-Jen Catholic Univ., Xinzhuang, Taiwan
Abstract: Time-frequency analysis techniques such as short-time Fourier transform and wavelet transform are used to analyze the neuronal dynamics of spike trains. However, because of the prerequisites of data continuity and time interval equalization, these techniques can be applied only to the continued neural signals. In addition, the recorded spike train processed using the spike-sorting algorithm is a point sequence of unequal time interval sampling, and well-known time-frequency methods cannot be directly applied. A typical strategy for transforming the time-point data from uneven to uniform sampling is the preprocess of binning or kernel fitting, which equalizes the intervals of data points. However, it is difficult to select a suitable bin size or kernel width. In the present study, we used the interspike interval to generate the amplitude of the point sequence to retain the uneven property, and we extended the application of the weighted wavelet Z-transform (WWZ) from astronomy to manage the uneven data. We also considered the effects of spike-sorting-induced errors in neural firing properties by using 6 spike sorting error models. We explored the tolerance of the WWZ to spike sorting errors in time-frequency data, and the results demonstrate that the WWZ is effective in processing sorted neuronal spiking trains. In conclusion, the WWZ is a state-of-art, promising tool for electroneurophysiological study.


Poster

543. Data Analysis: Neuronal Networks

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 543.21/DD27

Topic: G.07. Data Analysis and Statistics

Support: KAIST

Brain Korea 21 Plus Project 2015
Samsung Electronics
SK Telecom
NRF-2010-0018837
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Title: Modeling brain hierarchical structure using graph-based manifold learning
Hierarchical organizations of information processing in the brain networks have been known to exist and widely studied. However, there are some limitations on the main technique of the conventional methods, which extracts pairwise hierarchical relationships by utilizing observable anatomical criteria based on tract tracing experiments. Therefore, we need to design a new method computing hierarchy levels given the limited amount of hierarchical information. In this study, we suggest a new framework that can discover hierarchical structures of the brain networks with only the connectivity matrix and hierarchical information of a very few areas. Our strategy decomposes into three parts. In the first part, we use the graph Laplacian eigenmap which is one of graph-based manifold learning technique, to extract the character of each cortical area given the only adjacency matrix. Then the characteristics of each area are represented as a $k$-dimensional Euclidean vector. In the second part, we derive inherent pairwise relationship by using obtained $k$-dimensional vectors, and define pseudo hierarchical distances for every pair of areas. In the third part, we fix the hierarchy levels of only a few nodes, and compute hierarchy levels of areas by minimizing the objective function which is the sum of the square of the hierarchical distance error for all the pairs. We selected two brain connectivity data sets which are the macaque vision and somatosensory-motor networks. The hierarchical levels of the two data sets are known. We compared the results of our algorithm with Felleman & Van Essen (1991) model on the two data sets by plotting the linear regression line and the correlation between them, and our results were well fitted with the results of Felleman & Van Essen model. Thus, we can conclude that our method is able to capture the hierarchical organizations by using a small amount of information, and this study will help to investigate diverse brain hierarchical organizations.


Poster

543. Data Analysis: Neuronal Networks

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 543.22/DD28

Topic: G.07. Data Analysis and Statistics

Support: FCT grant SFRH/BD/79501/2011
Title: Bayesian model inversion of coupled mean-field integrate-and-fire populations: application to voltage-clamp currents and firing rates of CA1 neurons during gamma oscillations

Authors: *M. F. LEITE*¹, P. FIGUEIREDO², K. FRISTON³, D. KULLMANN⁴, L. LEMIEUX⁴;
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Abstract: The scientific investigation in vivo, in vitro, or via computational modelling of gamma oscillations has now a long history, yet it continues to attract great interest in the scientific community due to the critical role these oscillations play in active states of the healthy and diseased mammal brain (e.g. [1]). Here we introduce and showcase a new methodology that bridges the gap between these different investigative approaches. Namely, we build a mean-field population model of conductance-based integrate and fire neurons that is able to make specific predictions about both trans-membrane currents and neuron firing rates. When coupled, these population models can show a wide repertoire of behaviours. Here, we couple one population of pyramidal cells and one population of inhibitory interneurons in accordance with previous successful modelling studies of the mechanisms of gamma oscillations [2]. Further, we extend a Bayesian model inversion technique, Dynamic Causal Modelling [3], to handle dynamics in limit-cycle (or generally in closed orbit) regimes. We use prior parameter distributions for CA1 neurons from a public database [4]. We then use cycle averaged data recordings of voltage clamp currents at -70 mV and +10 mV, and firing rates from one sample pyramidal neuron and one inhibitory interneuron, showing firing rate phase coupling with the on-going carbachol induced gamma oscillation, in the CA1 region of a rat hippocampal slice preparation. The empirical data and predictions from the fitted model are presented in figure 1 and demonstrate good agreement. Following model inversion, one can now make predictions about new data independent of the fit, for example, inter-spike time interval (ISI) distributions, and identify the dominant mechanism and ways of modulating the underlying gamma oscillation (e.g. E-I vs I-I). [1] Akam et al. (2014) Nature Neuroscience [2] Brunel. (2000) Journal of Computational Neuroscience [3] Friston (2007) Neuroimage [4] www.neuroelectro.org (accessed Dec 2014)
Fig.: Fitted model predictions versus real data. Note the particularly good fits of +10 mV (inhibition) currents to the pyramidal cell or the inhibitory-interneuron firing rates. On the other hand, note as well the slightly poorer fit on the pyramidal cell firing rates.


Poster

543. Data Analysis: Neuronal Networks

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 543.23/DD29

Topic: G.07. Data Analysis and Statistics

Support: BMBF Grant FKZ: 01GQ1002
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Max Planck Society

Title: Correlations and signatures of criticality in neural population models

Authors: M. NONNENMACHER1,2, C. BEHRENS3,4,2, P. BERENS1,4,2,5,6, M. BETHGE1,3,4,2,*J. H. MACKE7,1,2;
1Max Planck Inst. for Biol. Cybernetics, Tuebingen, Germany; 2Bernstein Ctr. for Computat. Neurosci., Tuebingen, Germany; 3Ctr. for Integrative Neurosci., Tuebingen, Germany; 4Inst. of Theoretical Physics, Univ. of Tuebingen, Tuebingen, Germany; 5Inst. of Opthalmic Res.,
Abstract: Large-scale recording methods make it possible to measure the statistics of neural population activity, and thereby to gain insights into the principles that govern the collective activity of neural ensembles. One hypothesis that has emerged from this approach is that neural populations are poised at a thermodynamic critical point, and that this may have important functional consequences. Support for this hypothesis has come from studies that identified signatures of criticality (such as a divergence of the specific heat with population size) in the statistics of neural activity recorded from populations of retinal ganglion cells. What mechanisms can explain these observations? Do they require the neural system to be fine-tuned to be poised at the critical point, or do they robustly emerge in generic circuits? We show that indicators for thermodynamic criticality arise in a simple simulation of retinal population activity, and without the need for fine-tuning or adaptation. Using simple statistical models, we demonstrate that peak specific heat grows with population size whenever the (average) correlation is independent of the number of neurons. The latter is always true when uniformly subsampling a large, correlated population. For weakly correlated populations, the rate of divergence of the specific heat is proportional to the correlation strength. This predicts that neural populations would be strongly correlated if they were optimized to maximize specific heat, which is in contrast with theories of efficient coding that make the opposite prediction. Our findings suggest that indicators for thermodynamic criticality might not require an optimized coding strategy, but rather arise as consequence of subsampling a stimulus-driven neural population.


Poster

543. Data Analysis: Neuronal Networks

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 543.24/DD30

Topic: G.07. Data Analysis and Statistics

Support: National Institute of Mental Health (MH097822)

    Whitehall Foundation

    NARSAD
Hartwell Foundation  
Graduate Research Fellowship from the National Science Foundation  

Title: Network analysis of prefrontal cortical microcircuit dynamics after chronic stress hormone exposure and ketamine treatment  

Authors: *R. N. MODA*¹², J. WITZTUM³, C. LISTON²;  

Abstract: Chronic stress has been shown to alter neuronal morphology within the prefrontal cortex (PFC). This includes dendritic retraction and spine loss. Additionally, clinical studies have shown that low doses of ketamine, an NMDA antagonist, can act as a fast-acting antidepressant in treatment-resistant depressed patients. This is thought to be mediated in part by a ketamine-induced increase in postsynaptic dendritic spine density in PFC pyramidal cells. How changes in synapse number and dendritic morphology affect PFC microcircuit function is not well understood. To address this question, we investigated neural network dynamics in PFC microcircuits using two-photon calcium imaging. Mice were intracranially injected in the prefrontal cortex with a pan-neuronal GCaMP-expressing AAV virus, and a glass window was implanted in the skull for visualization of GCaMP-expressing cells. PFC microcircuit activity was quantified by two-photon calcium imaging before and after a chronic, 21-day exposure to corticosterone, the principal murine stress hormone, via corticosterone tablets that were implanted subcutaneously. The mice were then injected with an acute intraperitoneal dose of ketamine, and repeat imaging was performed 24 hours later from the same neural population. PFC microcircuit activity and network properties were analyzed, and results suggest that chronic corticosterone exposure reduces individual cell activity and functional connectivity in the PFC. Further, analysis of graph metrics of functional connectivity in PFC networks support the hypothesis that chronic corticosterone exposure reduces network efficiency, the strength of neural connections, and nodal clustering. Importantly, a single injection of ketamine partially modulates these deficits, resulting in increased cell activity and functional connectivity.  

Disclosures: R.N. Moda: None. J. Witztum: None. C. Liston: None.

Poster  

543. Data Analysis: Neuronal Networks  

Location: Hall A  

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM  

Program#/Poster#: 543.25/DD31
Title: Path-analytic Structural Equation Modeling to evaluate connections between primary sensorimotor cortical regions in chronic stroke

Authors: *K. JUNG¹, M. R. BORICH²;
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Abstract: Previous studies of sensorimotor control have demonstrated the primary motor and sensory cortices are dynamically coupled during movement. In chronic stroke, measures of cortical grey and white matter morphology have been correlated to altered cortical excitability and cortical connectivity. However, it is not yet evident how these regions are connected and influence one another to support sensorimotor control after stroke. Here, we employed a path-analytic structural equation modeling (SEM) approach for such a brain connectivity analysis, called path-analytic generalized structured component analysis (GSCA; Hwang & Takane, 2014). Path-analytic GSCA enables specification and estimation of the relationships among ROIs. For parameter estimation, path-analytic GSCA applies a least squares estimation method, and the standard errors of parameter estimates are estimated by the bootstrap method. As such, path-analytic GSCA is computationally efficient in dealing with more elaborate and complicated brain connectivity models without recourse to distributional assumptions. We applied path-analytic GSCA to a bi-directionally connected structural model of cortical thickness data extracted from four ROIs chosen a priori to investigate primary sensorimotor regions bilaterally (M1 Ipsilesional (i), S1_i, M1 contralesional (c), and S1_c) in participants with chronic stroke (n=8) and four ROIs (M1_Right (R), S1_R, M1_Left (L), and S1_L) in healthy controls (n=8). Then, we examined the difference between the stroke group and healthy controls on the structural paths in the hypothesized model. The result of our analysis revealed that there were statistically significant differences for the following structural paths: (1) bidirectional paths between M1_i and S1_i (controls: M1_R and S1_R); (2) a unidirectional connection from M1_i to M1_c (controls: M1_R and M1_L); (3) a unidirectional connection from S1_i to S1_c (controls: S1_R and S1_L); (4) a unidirectional connection from S1_c to M1_c (controls: S1_L and M1_L). These preliminary findings suggest there are differences in intra- and inter-hemispheric primary sensorimotor structural coupling. All participants with stroke demonstrated minimal-moderate motor impairment. Thus, the observed differences in structural connectivity may provide insights into the mechanisms and behavioral consequences of sensorimotor reorganization after stroke. Future work will apply Dynamic GSCA to time-series data to further delineate connections within the sensorimotor control network and the influence of reorganization of these connections on motor performance in individuals with stroke.

Disclosures: K. Jung: None. M.R. Borich: None.
**Title:** Statistics of brain functional networks: Classification and Testing

**Authors:** *D. Fraiman*, 1,2, N. Fraiman3, R. Fraiman4;  
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**Abstract:** It is common today to describe neuroimaging data by using network or graph theory. Nevertheless, techniques for statistical analysis of these networks are not very developed. We herein address some statistical problems associated with the time evolution of brain functional networks constructed from EEG, MEG, or fMRI data. The approach presented here is non-parametric, i.e. it does not rely on a particular network model. Natural notions of center, variance and a depth function for networks that evolve in time are introduced. This allows us to develop several statistical techniques including testing, supervised and unsupervised classification, and a notion of principal component sets in the space of networks. Among other things, the results presented are important for the development of new diagnostic methods based on brain functional or structural network data. We show some examples, as well as a real data example.

**Disclosures:** D. Fraiman: None. N. Fraiman: None. R. Fraiman: None.
Title: Noise assisted empirical mode decomposition and phasor analysis for the characterization and detection of oscillations in the local field potential

Authors: *J. M. MIKKILA;
Psychology, York Univ., North York, ON, Canada

Abstract: Oscillations in the local field potential of cortical and subcortical structures are inherently non-stationary and comprised of an unknown number of components. The most common methods for characterizing the power spectrum (PSD) of the various oscillatory components of the local field potential require assumptions which are inherently violated by the data in question [1]. Namely the discrete Fourier transform requires a signal be periodic in the sampling time, and therefore stationary, while analyses based on the analytic representation of convolved bases require there to be a single component within the filtered band. These assumptions are routinely violated by researchers in neuroscience, and the results of these analyses compromised. In the case of quantifying the PSD of a segment of non-periodic signal, energy is spread to other frequency bins in such a way that the actual peaks in power can cease to exist, and non-existent ones occur [1]. While in the case of time-domain analysis, co-existent near components become conflated and are not suitable to characterization by a solitary pair of phase and amplitude measures. In place of analysis utilizing the Fourier transform, we demonstrate a variant on the complete ensemble empirical mode decomposition (CEEMD) [2] hybridized with heuristics on improving the accuracy of the EMD [3]. While phasor analysis using the Fourier assumptions inherent in the inverse DFT are subject to Gibbs artifacts. We leverage an assumption free set of phasor analyses [4] which can provide a highly accurate measure of the power spectrum and constituent oscillatory components. This scalable design allows for parallelization of the noise assisted realization of the CEEMD on GPUs or computing clusters. Furthermore, without assumptions, or time windows of analysis, this procedure alleviates both time and frequency uncertainty bounds providing a lossless description of the signal in the finite and minimum number of component descriptors. [1] W. Klonowski. (2009). Everything you wanted to ask about EEG but were afraid to get the right answer. Nonlinear Biomedical Physics. [2] M. Torres et al. (2011). A complete ensemble empirical mode decomposition with adaptive noise. IEEE. [3] R. Rato et al. (2008). On the HHT, its problems, and some solutions. Mechanical Systems and Signal Processing. [4] T. Qian et al. (2005). Analytic unit quadrature signals with nonlinear phase. Physica D: Nonlinear Phenomena.

Disclosures: J.M. Mikkila: None.

Poster

543. Data Analysis: Neuronal Networks

Location: Hall A
Title: Functional graphical models of mouse visual cortex

Authors: *E. TARALOVA, R. YUSTE; Columbia Univ., New York, NY

Abstract: Despite a century of intense research, neuroscience still lacks computational models of visual stimuli and the spatio-temporal firing patterns they elicit in the visual system of animals or humans. Traditional imaging techniques like functional magnetic resonance imaging (fMRI) indirectly measure responses of large numbers of neurons (voxels). However, to understand how neural activity gives rise to cognition, we need to model how collections of hundreds of neurons engage in complex interactions over time. In contrast to whole-brain or micro scale imaging of a small number of neurons, new imaging methods like two-photon calcium imaging can record the firing of hundreds to thousands of neurons in living animals. Imaging the operation of neural circuits at this mesoscale will enable better understanding of the cortical information processing algorithms relating the activity of neurons to visual stimuli. We develop machine learning methods to decipher the neural code that links perception with the firing of neurons in the cerebral cortex. We propose novel mathematical models for holistic neuron data analysis that provide a framework for understanding the neural circuitry, which is unattainable with current single neuron methods. The proposed models are data-driven and capture the probabilistic conditional dependencies between the neural activity and the visual stimuli. We have conducted targeted neurophysiological experiments that interrogate the operation of mesoscale cortical computing circuits in awake behaving mice using two-photon calcium imaging. We record spontaneous activity and activity evoked by a database of visual stimuli ranging from drifting gratings, wavelets, composite textures and complex scenes. Using this neurophysiology data we train Bayesian graphical models to capture the spatio-temporal functional dependencies between neurons. The result is a compact representation that links visual stimuli to neural activity. We validate the models in the tasks of encoding (predicting) the neural activity and decoding (classifying) the visual stimuli. This work contributes novel machine learning tools for analyzing the functioning of neural circuits that are critical to advancing our understanding of the primary visual cortex. Discovering how the neural circuitry involved in visual perception works is an essential step toward understanding central visual pathologies and developing treatments. Furthermore, decoding the neural activity will bring us closer to bridging the gap between the firing of groups of neurons and perception, and, ultimately, cognition.
**Poster**

**543. Data Analysis: Neuronal Networks**

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 543.29/DD35

**Topic:** G.07. Data Analysis and Statistics

**Title:** Statistical estimates of neocortex semantical allocation breakdown

**Authors:** *L. SEYMOUR:
Persinvitro, LLC, Lake Barrington, IL

**Abstract:** Providing quantified statistical estimates of the semantical allocation of the neural microcircuit network in the neocortex have not been explored in the past. These statistics would unquestionably support both neuroscience research and commercial, information technology developments in personalizing products. A method and process are described in this presentation based on an integrated R&D landscape of the current state-of-the-art in computational neuroscience, big data analytics, artificial general intelligence, digital biomedical diagnosis repositories and pervasive multimedia and sensory data aggregation. The method is driven by an agreed upon and real-time updated ontology of concepts, the hierarchy of the concepts, the evolution of the configuration of the concept allocation in the neuronal network during the lifetime of the subjects, the aggregation of crowdsourced concept allocation statistics across a reference group of subjects, the contextual correlation between the lifetime use of the concepts and the multiple instantiation of any single concept across a number of physically different neural microcircuits. Initial estimates show that using several different alternative approaches of the aggregation processes and algorithms would lead to a consistent variance range in microcircuit capacity allocation needs for a specific portfolio of concepts. The variance improves as the solutions are enhanced for broader coverage. These results indicate that building software tools for scalable commercial strength coverage of the concept ontology and the entire neocortex capacity is feasible at the current state of the art in the underlying domains characterized here in the interdisciplinary landscape analysis.

**Disclosures:** L. Seymour: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); PersInVitro, LLC. F. Consulting Fees (e.g., advisory boards); Experian.
Title: Understanding the structure and origin of transcranial magnetic stimulation artifacts in electroencephalographic signals

Authors: D. FRECHE\textsuperscript{1}, N. LEVIT-BINNUN\textsuperscript{1}, J. NAIM-FEIL\textsuperscript{2,1}, M. RUBINSON\textsuperscript{2}, *E. MOSES\textsuperscript{2}; \\
\textsuperscript{1}Interdisciplinary Ctr. (IDC), Herzliya, Israel; \textsuperscript{2}Weizmann Inst. of Sci., Rehovot, Israel

Abstract: The concurrent use of transcranial magnetic stimulation (TMS) and electroencephalography (EEG) is emerging as a powerful tool to directly probe cortical excitability and connectivity of the brain. Currently, a critical limitation for the field of TMS-EEG research is the strong artifact evoked by the TMS pulses in the EEG recordings which may persist up to a hundred milliseconds after the pulse and mask underlying neural activity. Despite the developments of new hardware solutions, improved EEG amplifier technology and application of various artifact removal methods (ICA and PCA), the artifact may be suppressed but cannot be completely removed from the recordings. Insufficient artifact removal may hinder interpretation of cortical responses and especially prevent analysis of TMS-EEG data with network-based analysis tools. To address these limitations, we provide a description of the TMS-evoked artifact and clarify its main origin. A quantitative model is constructed, capable of generating and reproducing the TMS artifact. The dominant contribution to the artifact comes from the charge distribution on the electrodes and the structure of the scalp. While puncturing the skin and using abrasive electrode gel can reduce the artifact, this does not completely suppress it. Our Scalp Artifact Model can be used to subtract and correct for the dominant TMS-evoked scalp-related artifact in the EEG recordings. We demonstrate that the Scalp Artifact Model should be applied prior to other physiological artifact removal methods (e.g. ICA or PCA for eye blinks or muscle movement) and that it greatly advances our ability to index cortical excitability and connectivity of the brain with TMS-EEG. This novel artifact model opens the possibility to apply graph-theory analysis tools to investigate network dynamics and stability of the brain using TMS-EEG.
Title: Bringing knowledge to data: Visualizing coverage of the neuroscience data space in the Neuroscience Information Framework

Authors: *T. GILLESPIE*¹, A. E. BANDROWSKI², J. S. GRETHE¹, M. E. MARTONE²; ¹Neurosci., UCSD, LA Jolla, CA; ²Neurosciences, UCSD, La Jolla, CA

Abstract: What do we known about the brain? Currently, we cannot answer this question in a meaningful and concise way because much of information that we have is in a form that is inaccessible and difficult to digest algorithmically, i.e., the published literature. Since 2008, the Neuroscience Information Framework (http://neuinfo.org) has been aggregating data collected about the brain that is scattered across hundreds of different websites and databases and now searches across 200 databases comprising over 800 million records. The richness of the aggregated dataset allows us to ask more global questions about the state of neuroscience information. Here we use NIF’s aggregate data and knowledge sources to reveal knowledge gaps and biases in the neuroscience dataspace. We use the NIF semantic framework, a collection of community-based ontologies covering the major domains of neuroscience, e.g. neuroanatomy, to tag the aggregate data sources. A Python-based workflow was developed to replace a prototype built in Kepler that queries for each term in the ontology across all data sources. These queries are used to produce semantically-enhanced “heatmaps” indicating the frequency with which terms do or do not appear in the dataspace. These heatmaps provide a unique overview of representation of neuroscience concepts within and between sources. Because the ontologies are relatively well populated, they represent a proxy for the state of knowledge within any one subdomain, e.g., brain anatomy, or disease. A comparison of the representation of neuroscience concepts across all sources shows that these entities are not uniformly studied; rather, we have over-representation of some entities and under-representation of others. For example, brain
structures in the forebrain tend to be more heavily represented than those in the midbrain and hindbrain. Further we show where and at what level of granularity concepts cross between different data types and sources. These heatmaps provide a unique opportunity to represent what we are calling the “neuroscience data space”, that is, data that is easily accessed by machine. We are currently exploring reasons for under- and over-representation through comparison with the literature and data sources like the NIH RePORTER for funded grants. We are also examining possible sources of experimental bias, particularly within the neuroimaging domain. The heatmap generation workflow is currently being ported to a web interface so that anyone can generate heatmaps for their domain.


Poster

544. Data Analysis: Networks and Software Tools, other

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 544.02/DD38

Topic: G.07. Data Analysis and Statistics

Support: This work is supported by the Applied Mathematics Program within the Office of Advanced Scientific Computing Research of the U.S. Department of Energy under contract No. DE-AC02-05CH11231

Title: High performance computing web service for the analysis of local field potentials

Authors: *S. Mackesey*\(^1\), M. Prabhat\(^2\), G. Buzsáki\(^3\), A. Khosrowshahi\(^4\), F. Sommer\(^1\);
\(^1\)Univ. of California, Berkeley, Berkeley, CA; \(^2\)Lawrence Berkeley Natl. Lab., Berkeley, CA; 
\(^3\)New York Univ., New York, NY; \(^4\)Nervana, San Diego, CA

Abstract: High-density multielectrode recordings of neural activity offer unprecedented opportunities to understand the function of the brain. However, the dimension of the data often prevents the broad mining of the signal structure. For example, the multi-site local field potential (LFP) is still a largely unexplored frontier in neural coding. Successful recent approaches to extract information from the LFP combined unsupervised and supervised learning methods, e.g., Agarwal et al 2014. We propose to create a community web service offering the computing resources and the software framework for processing large neurophysiological datasets. The resource will use the Lawrence Berkeley National Labs’ NERSC (National Energy Research
Scientific Computer Center) supercomputing cluster, as well as the data format standard emerging from the Neurodata Without Borders (NWB) project. Here we present a proof-of-concept of this web service for a data analysis pipeline consisting of the following three steps: (1) filter to a band of interest; (2) apply unsupervised learning methods to the filtered data; (3) assess the correlation of learned features with stimulus, behavioral, or other physiological variables using supervised learning methods. A parallel implementation of convolutional sparse coding (CSC) for unsupervised learning was developed and optimized for running on the supercomputing cluster. The implementation plugs into a library that reads and writes dataset files structured according to the developing NWB data format standard. The features resulting from CSC, as well as their association with arbitrary behavioral, stimulus, or other physiological variables (e.g. position), may be visualized via a web interface. We demonstrate our toolset's utility using a 512-channel neurophysiology dataset captured via silicon probes embedded in the hippocampus of rats navigating a linear track. The raw LFPs were filtered into 4 frequency bands: 6-10 Hz (theta), 20-55 Hz (slow-gamma), 60-100 Hz (fast-gamma), and 20-100 Hz (broadband gamma). Sparse dictionaries for the data were derived using our CSC implementation. We quantified the localization of the resulting sparse features and used generalized linear models to assess their correlation with position.

Disclosures: S. Mackesey: None. M. Prabhat: None. G. Buzsáki: None. A. Khosrowshahi: None. F. Sommer: None.

Poster

544. Data Analysis: Networks and Software Tools, other

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Program#/Poster#: 544.03/DD39

Topic: G.07. Data Analysis and Statistics

Support: NIH Grant R01 DC009977

Title: Exploring data-driven techniques for visual representation of neuronal micro-connectomes

Authors: *L. MARENCO*1,2, R. Wang1, R. A. McDOUGAL2, T. M. MORSE2, N. T. CARNEVALE2, P. MILLER1, G. M. SHEPHERD2;
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Abstract: The connectivity between neurons in the brain, often referred to as connectomics, is driving much current research on fiber tracts between and interconnectivity within brain regions. This is creating the need for neuroinformatics support for databases for the neuronal data, and a
new generation of efficient digital representations of the interconnections and interactions underlying neuronal integration so that they can be both searchable by automatic search tools and extended as new data is obtained. SenseLab, an interoperable suite of databases dedicated to supporting research on dendritic properties and synaptic organization, is being adapted for these new connectomics challenges. Building on the SenseLab extensible data model, our group is increasingly incorporating new connectivity details at the neuronal compartment level. Part of this new information is currently described using new neuronal connectivity pages in NeuronDB. The complexity of this information poses challenges to creating new interfaces. Our solution to this problem has been to build an interface capable of exposing complex neuronal connectivity in a simple, yet extensible way, with the ability to accommodate new categories of data in the future. The new tool we are developing for this purpose uses data-driven techniques to render dynamic graphics via internet browsers. This application retrieves neuronal information from our soon to be released new versions of FunctionalConnectomesDB and NeuronDB. The categories of information used by this tool comprise: a) Microcircuit; b) Neuron: type (principal and interneurons), canonical form and compartments, and relative size; and c) Synapses: transmitter type released from a cell compartment (e.g. glutamate from an axon terminal) and receptor type activated from a cell compartment (e.g. NMDA receptor in a proximal dendrite). We are incorporating brain regions as well as region/layers in order to cluster these neuronal compartment connections. The tool is available at this URL http://ycni.med.yale.edu/PubLinks/HBP/2015SfNConDiagram


Poster

544. Data Analysis: Networks and Software Tools, other

Location:  Hall A

Time:  Tuesday, October 20, 2015, 8:00 AM - 12:00 PM
Program# Poster#: 544.04/DD40

Topic: G.07. Data Analysis and Statistics

Support: NSF 1406556

Title: Representation of depth electrodes using parcellated cortical surface maps

Authors: *M. ROLLO¹, C. M. KADIPASAOGLU¹, N. TANDON²;
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Abstract: The use of intracranial electroencephalography (icEEG) recordings in patients with pharmaco-resistant epilepsy is a common strategy in epilepsy surgery. This is generally conducted using subdural grid electrodes but the advent of neurosurgical robotic assistance has made stereotactic EEG (sEEG) using depth electrodes a broadly viable option. Currently, methods to localize the depth electrodes in imaging space are lacking. Precise localization is crucial when determining seizure foci and the representation of detected electrical fields is necessary to plan interventions after sEEG electrode placement. Here, we describe a novel method to precisely localize depth electrodes in patient specific imaging space. To localize the depth electrodes, the patient’s Axial Bone CT is first aligned with their T-1 weighted image using AFNI. We then use FreeSurfer to processes the T-1 weighted image and create parcellated cortical surface maps (FreeSurfer software). The parcellated cortical surface maps are then converted into masks for the left and right hemisphere via AFNI. The masks are applied to the co-registered CT to remove the skull and any artifacts outside of the skull. The masked CT scan is also thresholded so that only the artifacts representing the depth electrodes remain. Regions of interest (ROIs) are then created around the artifacts using AFNI’s 3dclust command, which determines the coordinates for the maximum intensity value in each volume cluster. These coordinates represent the center of each contact and are used to create a displayable object in SUMA. Thus, the depth electrodes are localized and can also be visualized in SUMA, along with the cortical surface reconstruction of the patient's brain. Additionally the prior cerebral parcellation can be used to isolate the gyral labels for each individual recording electrode as well as identify which electrodes are located in white matter, in semiautomatic fashion.

Disclosures: M. Rollo: None. C.M. Kadipasaoglu: None. N. Tandon: None.

Poster

544. Data Analysis: Networks and Software Tools, other

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM
Title: Atomizing data to ensure that experimental, analytical and/or administrative data points, are: tangible, useable, fixed and federatable (TUFF-data)

Abstract: Neuroscience data now generally exist within digital formats. This makes it easier to transpose data points from one format to another, or one location to another. During initial creation of a data point or its subsequent transformation or translocation, key information about that data point can be lost. For example, information about data identity or context and process that led to its creation can be lost making it harder to determine if the data is what it is supposed to be. Even if that information can be recovered, that process can be costly. Here we discuss the feasibility of atomizing data at its point of creation through uniquely identifying instantiation of any particular data point. By atomized data, we mean that each data point is uniquely identified and recognized in a manner dependent on its elemental digital properties regardless of its information content. For that purpose, it is necessary to explicitly define the concept of a data point as being composed of: 1) the value returned by an query or probe within a predefined framework and 2) documentation of information necessary and sufficient for evaluating whether that value is accurate and replicable (i.e. reproducible). In that sense, the actual value is like an atomic nucleus and its reproducibility documentation acts like an atomic electron cloud. Together, they provide the data point with substance and chemistry allowing this elemental unit of data matter to interact and be combined into larger more complex data objects. That chemistry should occur without changing the identity and integrity of the objects atomic components and should remain unchanged during recording, archiving or retrieval processes. Unlike physical atoms, the periodic table of atomized data-points can consist of any arbitrarily large address space represented by any valid number system. This poster will describe a general method for atomizing data in a way that can be implemented with commodity instrument components and commodity software. The method is demonstrated using a computer controlled consumer camera sensor (a Canon DSLR). A data value generated by an area of interest on that sensor is bundled up with reproduction documentation and stored in a data point archive file format like ZIP. This data point is then uniquely identified in a way dependent of that value and that documentation within that archive using an algorithm. The identifier remains the same no matter how many times the value and its documentation are accessed and interrogated. The approach atomizes data-points and makes them: tangible, usable, fixed and federatable (TUFF-Data). It also enables an Ato-Publication strategy for promoting open science and replication.

Disclosures: P.S. Pennefather: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Authors are sole
owners of gDial Inc that is commercializing software based on these ideas. **W. Suhani**: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Authors are sole owners of gDial Inc that is commercializing software based on these ideas.

**Poster**

544. Data Analysis: Networks and Software Tools, other

**Location:** Hall A

**Time:** Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

**Program#/Poster#:** 544.06/DD42

**Topic:** G.07. Data Analysis and Statistics

**Support:** NIH MH097366

NIH MH076188

**Title:** A new paradigm in accessing and analyzing big brain data

**Authors:** *J. KORICH*¹, N. J. O’CONNOR¹, P. J. ANGSTMAN¹, B. S. EASTWOOD¹, M. J. FAY¹, J. O. BLAISDELL¹, S. J. TAPPAN¹, K. E. DAY¹, H. J. KARTEN², C. R. GERFEN³, J. R. GLASER¹;

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**Abstract:** Recent advances in slide scanning technologies and sample preparation have ushered in a new era of big data in microscopic brain imaging. Clearing techniques that allow deeper imaging into intact tissues, and fast high-resolution whole slide scanning technologies result in data sets that easily exceed tens of terabytes of image data per brain. These big image sets introduce new challenges in image storage, organization, viewing, and analysis. To overcome these hurdles we developed a platform for storing, serving, organizing, viewing, and analyzing large-scale image data. The platform consists of three networked components: 1. high-performance image server software and database (Biolucida), 2. an image storage device, and 3. a portable image analysis suite (BrainMaker, NL360). The image analysis software supports the alignment and compilation of 3D images from full resolution serial sections automatically delineated from whole slide images, neuronal reconstruction and connectivity analysis, and cellular population estimation. Together, these methods provide previously unavailable high resolution measurements from big data images with anatomical context provided by visualizing the aligned 3D images. Here, we present three sets of brightfield whole slide images containing Giemsa stained series through Nile rat brain residing on a server (wiley.biolucida.net/images)
and accessed by our remote analysis tools from MBF offices in Vermont. Serial sections were extracted, aligned, and compiled into 3D images and rendered. For these reconstructions, estimations of the volume of cholera toxin-B tracer tracts through the brains were generated. Also, previously aligned and compiled fluorescent 3D images of mouse brain were served from the GENSAT collection (GENSATcreBrains.biolucida.net) and rendered in 3D. Automated estimates of cellular population statistics are also reported. These examples present our platform’s ability to remotely visualize and analyze whole slide image data in the forms of unprocessed serial sections and volumetric data. Increasingly large data sets from modern imaging methods (e.g., quantitative array tomography, light sheet microscopy, and clearing methods) necessitate a platform for managing, serving, and analyzing big data. Efficiently serving big neuroanatomic image data to remote analysis facilities alleviates the need to maintain and transfer multiple copies of image data that exceed typical storage, memory, and file format size limitations. It is our hope that it also opens the possibility of independent evaluation and open sharing of primary data by third-party observers.


Poster

544. Data Analysis: Networks and Software Tools, other

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 544.07/DD43

Topic: G.07. Data Analysis and Statistics

Support: NIH Grant R01 DC009977

NIH Grant T15 LM007056

Title: Unified real-time searching of keywords and attributes in ModelDB
Abstract: ModelDB is a public database that supports computational neuroscience by making models associated with publications available for download. Readily accessible model source code is critical for allowing simulation results to be verified and extended. By sharing code, ModelDB also acts as a common place to find examples of how to implement models for each of the approximately 70 different simulation environments represented in the database. ModelDB has grown to include the source code for approximately 1000 published computational neuroscience models. This large number of models has made it critical to provide search tools that assist researchers — both experimentalists and modelers — in finding models of interest. Previously we created a variety of search strategies (paper author name or model accession number, full text search, attribute (keyword) search, etc). Multiple search methods burdened the modeler with the decision of which search method to use. To simplify and accelerate searching, we (R.A.M.) replaced the multiple search fields on ModelDB's homepage with a single search box — available at the upper left of every ModelDB page — that combines full text, attribute, and model accession number searches. After a few characters are entered, the search box dynamically supplies possible completions and attribute search results with headers in a drop-down list. Attributes are considered to match if any part of the value matches the entered text; for example, a search for "memory" would suggest the model topic "working memory". Author suggestions include a secondary drop-down menu with each of that author's models; clicking on a model title opens the corresponding model. Full text suggestions list possible completions for the word being entered; if there are no completions shown for a 3+ letter word, then the entered characters do not begin a word in the repository, indicating a possible typo. The figure shows an example of the real-time search results. The new method greatly enhances the ability of researchers to find models directly relevant to their interests.

Disclosures: T.M. Morse: None. R.A. McDougal: None.
Title: Independent Component Analysis (ICA) for ocular artifact correction in EEG data is far from perfect: limitations and trade-offs

Authors: *J. DREO, B. PIKŠ, A. EMERŠič, Z. PIRTOŠEK;
Lab. For Cognitive Neurosci., Ljubljana, Slovenia

Abstract: Among the common EEG artifacts blinks represent one of the biggest challenges to quantitative analysis. This is because they occur very frequently, have large amplitudes compared to background EEG and also contaminate all scalp channels -albeit to differing degrees. Their efficient correction is thus paramount to most QEEG analyses. Independent Component Analysis (ICA) is a mathematical method of generating components that are linear combinations of the original EEG channels. While the resulting components do not necessarily have any a priori relation to physical sources, it is often noticed that they are similar to known source signals (muscular, alpha wave, eye-movements⋯). Excluding components that likely contain blinks, before re-assembling the rest, effectively subtracts blinks from EEG data. But since no component is truly “clean” (free of any other type of source activity) removing blinking-related components will also remove at least some genuine EEG signals. We conducted a basic review of 147 recently published EEG papers (2015-2012) and found that of those using and adequately reporting on ocular artifact correction (OAC): 58% used ICA, 25% a regression method, 11% source analysis and 6% used other methods. Since ICA seems to be the most popular method of OAC, assessing how effective it is and also how might corrupt EEG data is crucial. We assessed the effect of A) two ICA algorithms (Infomax, FastICA), three input data lengths (300, 600, 900 sec) and C) 6 different levels of component exclusion (1, 2, 3, 4, 5 and an adaptive method) on the efficiency of OAC and EEG preservation. The former was quantified by averaging 1 sec segments surrounding blink peaks after their removal by ICA. The average amplitude remaining after ICA on frontal EEG channels in the interval [-150,+150] ms relative to blink peaks was used as a measure of remaining blink activity. We quantified the preservation of background EEG activity by the remaining P3 amplitude in an Oddball task after ICA. These analyses were performed on 37 healthy subjects using 64-channel EEG data sampled at 500 Hz. Our results show that even when rigorously selecting which components to exclude based on correlation analysis, ICA still leaves between 2-20 µV of blinking-related activity. Trying to reduce these residual artifacts by excluding more components (>3) seriously degrades EEG activity as measured by decreases of P3 amplitudes on the order of 10-50%. We thus conclude that ICA, while often employed, is far from a perfect method of OAC. It is a sub-optimal trade-
Disclosures: J. Dreo: None. B. Pikš: None. A. Emeršič: None. Z. Pirtošek: None.

Poster

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Program#/Poster#: 544.09/DD45

Topic: G.07. Data Analysis and Statistics

Support: NIH Grant U01NS090569

Title: Suppressing false neural signals: Using object-tracking for motion compensation while imaging in the mouse cortex

Authors: W. LOSERT\textsuperscript{1}, *D. E. WINKOWSKI\textsuperscript{2}, E. MARSHALL\textsuperscript{1}, M. J. HARRINGTON\textsuperscript{1}, P. O. KANOLD\textsuperscript{3}; \textsuperscript{1}Physics, Univ. of Maryland, College Park, MD; \textsuperscript{2}Inst. Systems Res., \textsuperscript{3}Biol., Univ. Maryland, College Park, MD

Abstract: The application of 2-photon laser scanning microscopy (TPLSM) techniques to measure the dynamics of cellular calcium signals in populations of neurons is an extremely powerful technique for characterizing neural activity within the central nervous system. Moreover, the use of awake and behaving subjects is becoming necessary in order to understand how neural circuit elements cooperatively interact to form sensory perceptions and generate behavior. A major challenge in imaging such preparations is the relative motion between the imaging location and the microscope due to animal and tissue movement. Although there are surgical and technical approaches to minimize brain motion under these conditions, it is generally unavoidable. This relative movement creates presence of image jitter at different times within the acquired image sequence. The presence of image jitter, particularly in the Z-dimension, is highly problematic when trying to quantify fluorescence signals from small regions of interest (ROIs) within the image sequence. Here, we used TPLSM to image either a) static signals from fluorescent proteins expressed in subtypes of cortical neurons or b) dynamic signals from genetic encoded calcium indicators in the superficial layers of auditory cortex of anesthetized or awake mice. In addition, we imaged in single and across multiple focal planes (i.e., volumes) of cortical tissue. To address the problem of brain motion, we have developed a procedure of motion compensation that relies on locating neurons and tracking their positions...
over time. Object tracking is accomplished using established techniques of image processing that we have adapted from the field of soft matter physics. We describe the performance of our techniques both for 2D (single plane) and 3D (volume) image sequences. For comparison, we also present the performance of widely used image registration techniques. Our analysis demonstrates the benefit of correcting for vertical motion across focal planes, as a means of suppressing false neural signals.


Poster

544. Data Analysis: Networks and Software Tools, other

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program#/Poster#: 544.10/DD46

Topic: G.07. Data Analysis and Statistics

Title: A comparison of motion correction techniques applied to functional near-infrared spectroscopy data from children

Authors: *X. Hu*1, M. M. Arredondo2, N. Confer2, A. Dasilva3,4,1, M. Shalinsky1, I. Kovelman2,1; 1Ctr. for Human Growth and Develop., 2Dept. of Psychology, 3Sch. of Dent., 4Headache & Orofacial Pain Effort Lab., Univ. of Michigan, Ann Arbor, MI

Abstract: Functional near-infrared spectroscopy (fNIRS) is an emerging optical brain imaging technique, and is becoming an increasingly popular method for child brain imaging. fNIRS measures the hemodynamic changes that effectively reflect brain activities occurring while people perform a wide range of mental tasks; it can provide both topographic and tomographic brain images. Motion artifacts are significant sources of noise in functional near-infrared spectroscopy (fNIRS) data, especially in studies with child and infant participants. In fact, motion artifacts are one of the most significant concerns in pediatric brain imaging designs and data analyses. Different methods have been developed to correct motion artifacts in fNIRS data, but the relatively effectiveness of these methods towards pediatric data which is often found to be significantly noisier than adult data remains largely unexplored. The issue is further complicated by the heterogeneity of fNIRS data artifacts. In the current study, we compared the efficacy of the six most prevalent motion artifact correction techniques with fNIRS data acquired from children participating in a language acquisition task, including wavelet, spline interpolation, principal component analysis (PCA), moving average (MA), correlation based signal
improvement (CBSI) and combination of wavelet and moving average. We evaluated five predefined metrics (including the motion artifact quantity variation, t-values and R-values of general linear model regression, the area under the hemodynamic curves from 0-1.5 seconds and 1.5-3.0 seconds) to quantitatively evaluate the performance of the different motion correction methods. The comparison results suggest that the moving average and wavelet methods yield the best outcomes. These findings elucidate the varied nature of fNIRS data artifacts, the efficacy of artifact correction methods with pediatric populations, and will help inform both the theory and practice of optical brain imaging.


Poster

544. Data Analysis: Networks and Software Tools, other

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 544.11/DD47
Abstract: Comprehensive knowledge of how brain areas and neurons are connected at both meso- (region-by-region) and micro-scales (synapse-by-synapse) is essential for understanding brain functions. Guided by the established brain-wide online meso-scale connectome resources (www.mouseconnectome.org and http://connectivity.brain-map.org), we developed a platform for further detailed sub-region and synapse-level circuit mapping in basal ganglia circuitry. We obtained meso-scale dataset from injections and projections of fluorescent protein-expressing viral tracers and micro-scale dataset from mammalian GFP reconstitution across synaptic partners (mGRASP)-assisted circuit mapping (mGRASPing). We built a system that can reliably extract wiring information from digitized meso- and micro-scale images and reconcile these data into a hierarchical structure in a common 3D reference space. The system features 2D brain slices alignment, 3D registration or co-registration with reference atlas and proof editing. To achieve the high accuracy of registration required for sub-region connectivity mapping, anatomical annotations from registration were converted to splines and manually corrected if necessary. We believe that comprehensive cross-referencing of connectivity data from different scales into the same reference space will allow for exploring complex neuronal networks at multiple scales and will facilitate understanding circuit functions.


Authors: *S. WANG, W. MA, J. SI;
Arizona State Univ., Tempe, AZ

Abstract: M-Sorter was first introduced in 2012 (Yuan et al., 2012) aiming at automatic sorting of action potentials (spikes) based on raw neural waveforms. Potential spike detection in M-Sorter was based on our own multiple correlation of wavelet coefficients (MCWC) algorithm (Yang et al., 2011), which was followed by a template matching for spike clustering. A major update took place in M-Sorter2014 where spike clustering was carried out by our new accurate and robust expectation-maximization (AREM) algorithm. In this study, we present the 3rd generation M-Sorter, M-Sorter2015, which takes advantage of improved MCWC and AREM. Also in the new version, we developed an accurate and robust variational Bayesian (ARVB) approach for automated clustering as an alternative. The M-Sorter2015 consists of three major modules: 1) spike detection using improved MCWC; 2) feature extraction function by principle component analysis, independent component analysis, Wavelet-based analysis, Laplace Eigenvalue, or Relieff algorithm; and 3) automatic clustering by AREM or ARVB. Only two easy-to-choose free parameters, i.e. the noise floor level $\tau$ and the spike quality level S, need to be determined prior to using M-Sorter2015. The parameter $\tau$ for the namesake is the noise floor level from recorded signals. The spike quality level S reflects signal-to-noise ratio. Recommended S values are provided in the M-Sorters and were tested to show its robustness. The M-Sorter2015 was comprehensively tested and compared with M-Sorter2014, Klutakwik (Harris et al., 2000), Wave Clus (Quiroga et al., 2004) and Signal Energy detection plus T-Distribution EM clustering in Plexon’s Offline Sorter (Plexon, Inc.). Sorting results from artificial datasets (Wave Clus software package) and labelled real datasets (Harris et al., 2000; Henze et al., 2000) demonstrated superior performance of M-Sorter2015 over all compared sorters. Results by M-Sorter2015 with AREM or ARVB had lower false alarm rates and higher accuracy than other sorters. The following measures were used in comparisons: 1) results consistency or repeatability, 2) accuracy in determining cluster numbers under conditions of poor SNR, high similarity among clusters, and overlapping spikes. Comparisons were also conducted using real datasets (Yuan et al., 2015; Mao et al., 2015). Sorter performance was examined by waveform shapes, spike counts, waveform variances of sorted spikes, and interspike intervals. Results showed that M-Sorter2015 was at least comparable to other sorters.

Disclosures: S. Wang: None. W. Ma: None. J. Si: None.

Poster

544. Data Analysis: Networks and Software Tools, other

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM
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Topic: G.07. Data Analysis and Statistics

Support: Academy of Finland postdoctoral fellowship for Mikhail Paveliev
the Russian Government Program of Competitive Growth of Kazan Federal University for Mikhail Paveliev

Title: Quantification of spatial gradients in cells and tissues

Authors: *M. N. Paveliev¹,², N. Lipachev², N. Arnst¹, N. Kulesskaya², M. Saarma³, H. Rauvala²;

Abstract: Biochemical processes are spatially structured and compartmentalized both at the single cell and tissue levels. Tools are required for the quantification of the spatial distribution of biological molecules and cellular structures under normal and pathological conditions. Here we present two algorithms for the quantification of spatial gradients in single cells and in the spinal cord tissue. We propose a border-based contour segmentation method to quantify intracellular molecule distribution in cell microscopy images based on the distance from the plasma membrane. We use this method to quantify: 1) intracellular distribution of phosphorylated extracellular signal-regulated kinases 1/2 in confocal stacks in response to nerve growth factor and epidermal growth factor; 2) precursor form of glial cell line-derived neurotrophic factor in electron microscopy images; 3) spatio-temporal propagation of the intracellular Ca²⁺ response to glutamate in neurons. To quantify spatial gradients at the tissue level we propose an automated software tool measuring intensity and object size distribution around the hemisection injury site in the rodent spinal cord. We analyze the size and intensity of perineuronal nets (PNN) - the extracellular matrix structures that play an important role in the plasticity of the central nervous system. We demonstrate that the distribution of the PNN parameters is affected by the distance from the trauma epicenter and PNNs at the injury site differ from those in the uninjured tissue. Our results support the importance of the spatial distribution of biochemical processes at the cell and tissue levels under the normal and pathologic conditions.


Poster

544. Data Analysis: Networks and Software Tools, other

Location: Hall A
Title: Live-cell high-content analysis of synapse loss and recovery during HIV-1 neurotoxicity

Authors: *R. SPINDLER, K. A. KROGH, S. A. THAYER; Pharmacol., Univ. of Minnesota, Minneapolis, MN

Abstract: Here we describe a high-content analysis method that quantifies changes in excitatory synaptic connections between primary rat hippocampal neurons \textit{in vitro}. A key feature of the assay is the ability to track synapses from the same neuron over time. Thus, the assay can quantify the formation of synapses during development of the neuronal network, loss of synapses during neurotoxic insult, and drug-induced recovery of synapses following loss. Primary hippocampal neurons were cultured in 96-well plates and transfected with plasmids that encode the fluorescent proteins tdTomato and PSD95-GFP. In each of the 96-wells, a stack of 8 confocal images was acquired in 5 fields per well using an automated spinning disk confocal microscope. The stage position of each stack was recorded, enabling return to the same positions at later times. Automated image analysis was developed using MetaMorph software that identified postsynaptic densities as green fluorescent puncta in contact with a binary mask created from the red fluorescence image. Cognitive decline correlates with synaptodendritic injury in many neurodegenerative diseases, including human immunodeficiency virus-1 (HIV-1)-associated neurocognitive disorders (HAND). Because HIV-1 does not infect neurons, synapse loss in HAND results from the release of neurotoxins from infected cells, such as the HIV-1 protein transactivator of transcription (Tat). Application of Tat produced significant synapse loss following 16 h exposure. Addition of an antagonist for GluN2B-containing NMDA receptors ifenprodil (at t=16 h), induced a significant recovery in the number of synapses by 24 h. Thus, the automated acquisition and analysis assay successfully quantified the neurotoxic loss and drug-induced recovery of synapses. This high-content analysis should prove useful for screening a library of pharmacologically active compounds that induce the recovery of synapses following neurotoxic loss.

Title: A voxel-based morphometry pipeline in a computer cluster environment


Abstract: Introduction Anatomical phenotyping provides important biomarkers in mouse models of disease. Regional differences for anatomical regions defined in reference atlases (e.g. Waxholm) can be quantified after spatial normalization. Analyzing the deformation fields provides insight into morphometric change, and applying them to magnetic resonance diffusion tensor imaging (MR-DTI) parameters provides insight into microstructural changes. We present an efficient pipeline for regional and voxel based analysis, implemented in a high-performance computing environment. Methods The pipeline is scripted in Perl and runs on a cluster consisting of six nodes using the SLURM resource management software. We use MATLAB and Advanced Normalization Tools (ANTS) for data cleaning and registration to achieve a minimum deformation template (MDT). VBM analysis is performed using SurfStat. Label-based statistics are calculated via MATLAB. The primary functions of the pipeline have been validated using DTI images of the mouse brain, namely fractional isotropy (FA) and diffusion-weighted imaging (DWI). We assessed the ability of VBM in detecting known differences in FA and morphology through simulations. In select white matter structures FA values were reduced by ~12%. Morphologic changes in other regions were achieved by diffeomorphically registering a mask of a structure’s label to a version of itself that has been dilated or eroded by 1 voxel. VBM analysis was performed for a simple cross-sectional analysis using the FA and the log Jacobian images. The results were evaluated for three different sets registration parameters. Results We have tested the pipeline using both simulations and a mouse model of epilepsy. We assessed accuracy against manual segmentation for a mouse injected with kainic acid exhibiting a large hippocampus deformation. Dice coefficients demonstrated good agreement with manual labels: 82.5 (corpus callosum), 85.0 (anterior commissure), 91.9 (hippocampus) and 94.7% (caudate putamen). Discussion A critical step in voxel-based morphometry (VBM) is the creation of a minimal deformation template (MDT). This computationally intensive task requires a large number of pairwise diffeomorphic registrations, and is usually the rate-limiting factor in VBM.
Using a study specific MDT as intermediary between each image and the atlas produces more accurate segmentations compared to labels generated by direct registration to the atlas. Moreover, our automated VBM processing pipeline takes advantage of the parallel nature of a computing cluster, reducing the computation time from 16 to 2 days for a study with 9 control and 6 treated subjects, for example.


Poster

544. Data Analysis: Networks and Software Tools, other

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

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Topic: G.07. Data Analysis and Statistics

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DARPA N66001-14-1-4028 (GRAPHS)

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Title: Web visualization of massive neuroscience datasets using the open connectome project

Authors: *A. D. BADEN*¹, K. A. LILLANEY¹, W. GRAY RONCAL³, J. T. VOGELSTEIN², R. BURNS¹;

Abstract: Advances in neuroimaging techniques such as serial electron microscopy (EM), array tomography (ATomo), optophysiology (e.g. calcium imaging), CLARITY, and multimodal magnetic resonance imaging can yield data sizes exceeding workstation storage and working memory. For example, the Open Synaptome Project (http://opensynapto.me) expects to generate 1 PB of data over 5 years (~650 GB per day) using an automated ATomo pipeline. Visualization of these datasets is important both for quality assurance (e.g. confirming registration of spatial data) and for analysis. However, the massive size of each dataset precludes researchers from simply downloading data to their computers and using existing visualization tools. We have built a web-based neuroimaging visualization tool, ocpviz, which runs in modern browsers on a wide
variety of devices and supports a range of viewing options to allow neuroscientists to browse massive datasets on demand without having to store data locally. We designed ocpviz to get data from the Open Connectome Project (OCP, http://openconnecto.me) leveraging OCP’s RESTful web-based query system and cache hierarchy, which speeds up image load times. We support pan and zoom navigation, as well as keyboard shortcuts for zoom and for navigating between slices. Supported data types include 8, 16, 32, and 64 bit 2D, 3D, and 4D data, which collectively support raw images, segmentations (with false coloring), multi-channel datasets (e.g. ATomo), and time series datasets (e.g. calcium imaging). We will also support layering multiple data types with opacity controls for each layer. In addition to visualization of the raw image data, we provide query support for looking up metadata associated with visualized objects. For example, when viewing an annotated EM dataset (as in the figure below), the user can pull up a unique identifier for each annotation by clicking anywhere in the annotated region. This approach can be extended to any spatial queries supported by OCP, and allows us to integrate tools that operate on points in space.


Poster
544. Data Analysis: Networks and Software Tools, other

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

Program/#Poster#: 544.17/DD53

Topic: G.07. Data Analysis and Statistics

Support: NSF-ECCS12081804

Title: Automatic and accurate spike clustering based on robust variational Bayesian method
Abstract: We introduce a new cluster number determination scheme that can be integrated into robust variational Bayesian (RVB) algorithm and other automatic clustering methods. As a result, we were able to develop an improved automatic neural action potential (spike) clustering algorithm, referred to as automatic RVB or ARVB, with good properties such as improved accuracy, robustness, and consistency. The ARVB is implemented by two interleaving steps: 1) optimization of clustering once given cluster number using the RVB (Takashi et al., 2009); 2) cancellation of spurious clusters by unlabeled a cluster with minimum impact on clustering performance. The ARVB is initialized by the K-means with larger cluster number than actual, and then reduced automatically according to the interleaving two-step approach until a negative change of the ensemble likelihood, or the clustering performance measure, is above a threshold. Four different types of neural spike datasets were used for performance evaluation of the ARVB: simulated dataset with t-distributed clusters, artificial dataset from Wave Clus software package (Quiroga et al., 2004), labelled real dataset from Buzsaki lab (Harris et al., 2000; Henze et al., 2000), as well as labelled and unlabeled real data from our lab (Yuan et al., 2015; Hongwei et al., 2015). First, our evaluation results by using simulated and artificial datasets show that the ARVB clustering results were consistent over a reasonably wide range of parameter values under various data conditions. We then conducted extensive comparisons of ARVB with our own accurate robust expectation-maximization (AREM) algorithm developed in 2013, and three other popular sorters: Klutakwik (Harris et al., 2000), Superparamagnetic Clustering in Wave Clus (Quiroga et al., 2004) and T-Distribution EM clustering in Plexon’s Offline Sorter (Plexon, Inc.). AREM and ARVB provided comparable results to the three other algorithms measured by total classification accuracy and misclassification rate for datasets with high SNR and low similarity among clusters. But when comparing performance based on artificial datasets and labelled real datasets under challenging conditions (e.g. low SNR, high similarity), AREM and ARVB outperformed other classifiers. Furthermore, the ARVB at times outperformed our AREM. When evaluating classifier performance for real neural waveforms, four statistical measurements (J3, Pseudo-F, Davies-Bouldin and Dunn) and spike statistics were used. Both AREM and ARVB were at least comparable to, or sometimes subjectively better than the other three algorithms.

Disclosures: W. Ma: None. J. Si: None.

Poster

544. Data Analysis: Networks and Software Tools, other

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM
**Title:** ilastik - a software framework for interactive volume neuro-image analysis and for automatic calcium imaging analysis

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**Abstract:**

The interactive learning and segmentation toolkit (ilastik) is a general purpose pipeline framework for building modular, user-friendly biological image analysis applications. Its ability to handle and visualize image data in up to 5D and to perform fast calculations makes it especially attractive for neuroscience applications dealing with very large and diverse data. We present the general architecture of ilastik and a few easy-to-use workflows which accomplish the following tasks: 1. Synapse and membrane detection in Electron Microscopy (EM) volume images by interactively trained classification. For these workflows the user defines a set of possible semantic categories, such as synapses, membranes, or mitochondria and ilastik tries to predict the best matching category for each pixel or object in the volume. As soon as the user provides the first labels ilastik starts predicting the categories allowing fast interactive refinement of the classification results. The classification can be based on: 1.1 pixel-level labels and features [1, 4], 1.2 labels given to entire objects, as opposed to single pixels, and object-level properties such as shape. We show an application of this workflow to synapse and membrane detection in mammalian neural tissue volumes obtained by FIB/SEM and ssTEM microscopy. 2. Semi-automatic extraction of single processes in EM and LM volume images [3] based on inside-outside user annotations and segmentation uncertainty feedback. We show an application of this workflow to FIB/SEM volume image segmentation. 3. Automated Calcium Imaging analysis [5] allowing end-users to conveniently analyze their data with minimal manual effort. Its automated detection of cell centroids relies on a flexible matrix factorization that exploits the sparseness of neuronal activity in space and time. For CI analysis we also provide a new algorithm as an external package for ROI detection and spike train inference in a unified formulation. It allows finding an extremely sparse representation for sequences in terms of cell locations, cell shapes, spike timings and impulse responses [6]. Solving a single optimization problem yields cell segmentations and activity estimates without the need for heuristic pre- or postprocessing. Applications are composed of re-usable building blocks merged into workflows. For the GUI ilastik provides a flexible volume viewer component which supports pixel and object labeling. ilastik is available as open source software at www.ilastik.org. [1] C.Sommer et al, ISBI 2011 [2] B.Andres et al, ECCV 2012 [3] C.Straehle et al, CVPR 2012 [4] A.Kreshuk et al, PLoS ONE, 2011 [5] Diego et al, ISBI 2013 [6] Diego et al, NIPS 2014
Abstract: Purpose: Intracranial ERPs from epilepsy patients are a great opportunity to study the brain activity of cognitive processes with a unique spatial and temporal resolution. In this cases is of critical importance to know the exact localization of the electrodes. Here we present an open source Matlab toolbox (https://sourceforge.net/projects/ielectrodes/) to obtain the coordinates and anatomical labels of each electrode in a semiautomatic way with minimal user intervention. Method: Six patients implanted with grids and depth electrodes were studied. Post implantation T1 MRI and CT were coregistered using an affine transformation with SPM8 toolbox. Subject-specific cortical segmentation labels were obtained using Freesurfer software. Brain masks were obtained using FSL-BET software. T1 MRI and CT were coregistered to the MNI-152 space using a brain mask and nonlinear warping deformations with SPM8. Images were then processed in the toolbox (see figure). Electrode voxels were detected by using a dilated brain mask and thresholding high intensity CT voxels in a 3D reconstruction [1]. User intervention was needed to determine the threshold and dilation levels. Then, these voxels were
automatically clustered and the electrode coordinates were obtained. Electrodes were numbered and an anatomical label was assigned automatically to each one. A 3D view of electrodes over a semitransparent brain was shown for visual interpretation. CT and MRI sagittal, coronal, and axial views of electrode coordinates were visually checked by an expert. Electrode coordinates are finally exported to EEGLAB. Results: Six patients were studied with 104/332 SEEG/ECoG electrodes. In all cases, electrode coordinates were successfully located within visualized electrodes artifact in CT and MRI images. Conclusions: The proposed tool is a useful instrument to achieve a fast and robust localization and labeling of electrodes. [1] Blenkmann et. al. Grid and depth intracranial electrodes localization in a normalized space using MRI and CT images. IFMBE Proceedings 49 (2015): 413-416


Poster

544. Data Analysis: Networks and Software Tools, other

Location: Hall A

Time: Tuesday, October 20, 2015, 8:00 AM - 12:00 PM

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Title: Current source density method for single neurons

Authors: *D. CSERPAN1, Z. SOMOGYVARI1,2, D. WOJCIK3, H. GLABSKA3;

**Abstract:** The recent blooming of multi-electrode array (MEA) technology made it possible to measure extracellular electric potential with spatial and temporal resolution of tens of micrometers and several tens of kHz in thousands of points simultaneously. So far, however, these advancements seem to have mainly quantitative impact, no significant qualitative changes in experimental or analytical methodology have occurred, except for higher density of probing for spiking cells. Here we present two methods of data analysis utilizing these high density extracellular recordings for estimating detailed spatio-temporal description of electrophysiological processes on single cell level. These methods differ in their requirements and predictive power. If just the recordings of extracellular potential are available, spike Current Source Density (sCSD) method [Somogyvári et al., 2012] allows one to estimate profile of current sources from spike-triggered averages of measured potentials. If in addition morphology of the cell is available, which is viable in combination of MEA electrophysiology, patch-clamping and optical imaging, single cell kernel CSD (skCSD) method, an application of kernel CSD method [Potworowski et al., 2012] to this situation, allows one to reconstruct detailed spatio-temporal distribution of current sources along the cell at any moment in time. By applying these methods to simulated and experimental data we demonstrate their utility, study their limitations and applicability on several examples.

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**Poster**

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**Title:** Critical problems applying Granger causality analysis in neuroscience

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**Abstract:** Granger causality methods analyze the flow of information between time series. The Geweke measure of Granger causality (GG-causality) has been widely applied in neuroscience because its frequency-domain and conditional forms appear well-suited to highly-multivariate
oscillatory data. Here, we analyze the statistical and structural properties of GG-causality in the context of neuroscience data analysis. We analyzed simulated examples and derived analytical expressions to demonstrate how computational problems arise in current methods of estimating conditional GG-causality. We found that the use of separate full and reduced models leads to either large biases or large uncertainties in the causality estimates, and high sensitivity to uncertainties in model parameter estimates, producing spurious peaks, valleys, and even negative values in the frequency domain. We also analyzed how the generative system’s properties and frequency structure relate to the structure of GG-causality estimates. We used simulated examples and derived analytical expressions to show that GG-causality is independent of the receiver dynamics, i.e. the dynamics of the effect node that “receives” the input of the putatively causal node. In particular, the magnitude of the receiver response is ignored by GG-causality. This is potentially misleading in many neuroscience applications, where often the “cause” of a particular “effect” is being sought—for example, the foci of epileptic seizures—because from the perspective of GG-causality, the magnitude of the effect response is irrelevant. In addition, we found that GG-causality combines transmitter and channel dynamics in a way that cannot be disentangled without evaluating the component dynamics of the full model estimate. The separate-model fit computation in GG-causality leads to either large bias or large uncertainties that make the interpretation of frequency-domain structure highly problematic. Even if these computational issues are overcome, correct interpretation of GG-causality values is challenging, since GG-causality ignores receiver dynamics and is not informative of the system dynamics without consideration of the full model estimate. Our work suggests that GG-causality analyses could be easily misinterpreted without careful consideration of these factors. Through this work we hope to provide conceptual clarification of GG-causality and place it in the broader framework of modeling and system analysis, which may enable investigators to better assess the utility and interpretation of such methods.

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